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The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye

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Summary. Rye secalins are a polymorphic mixture of polypeptides which are classified into four major groups. Previous studies have shown that the structural genes for two of the groups (the ω-secalins and 40K γ-secalins) are located on the short arm of chromosome 1R and those for a third group (the high molecular weight secalins) on the long arm of the same chromosome. Analysis of F_2 grain from crosses between inbred lines of *S. cereale* shows that the structural genes for the ω-secalins (designated *Sec 1*) and the high molecular weight secalins (designated *Sec 3*) are loosely linked ($40.8 \pm 3.76\%$ recombination, 57.4 ± 11.30 cM). Analysis of wheat rye addition lines shows that the structural genes for the 75K γ-secalins are present on chromosome 2R. This locus is provisionally designated *Sec 2*. These genes are probably derived from those for the 40K γ-secalins by duplication, divergence and translocation. Analysis of secalin fractions from wild species of rye shows that all contain 75K γ-secalins, indicating that the duplication and divergence, if not the translocation, occurred before speciation of the genus.

Key words: Rye secalins – Structural genes – *Secale* sp.

Introduction

The alcohol soluble (prolamin) storage proteins of rye, called secalins, account for about 50% of the total nitrogen of the mature seed (Shewry et al. 1983c). They are a complex and highly polymorphic mixture of polypeptides which have been classified into four major groups. These are called the high molecular weight (HMW) secalins (M_r by sodium dodecylsulphate polyacrylamide gel electrophoresis of about 100,000 or greater), M_r 40,000 (40K) γ-secalins , M_r 75,000 (75K) γ-secalins and ω-secalins (M_r about 50,000). These groups, or components of them, have been purified and characterized and shown to be structurally related to groups of prolamins present in the related cereals barley and wheat (Charbonnier et al. 1981; Shewry et al. 1982; Field et al. 1982; Kasarda et al. 1983).

The chromosomal locations of the structural genes for secalins have previously been investigated by comparing the protein patterns of seed of wheat, rye, and lines of wheat with added chromosomes or chromosome arms of rye. This showed that structural genes for HMW secalins (sometimes called HMW glutenin) are located on the long arm of chromosome IR, with those for ω-secalins and the 40K γ-secalins on the short arm (Shepherd 1968, 1973; Shepherd and Jennings 1971; Bernard et al. 1977; Lawrence and Shepherd 1981). These locations are similar to those of the structural genes for the homologous prolamins of barley and wheat (Shewry et al. 1984). The 75K γ-secalins had not been characterized at the time of these studies and the location of their structural genes was not reported. Also, the numbers and linkage relationships of the controlling loci are not known.

In the present paper we report that the structural genes for the 75K γ-secalins are located on chromsome 2R, and also show that the loci on chromosome 1R which control the HMW secalins and ω-secalins are loosely linked. These results are discussed in relation to the locations and linkage relationships of the structural genes for prolamins in barley and wheat.

Materials and methods

Seed material

Seeds of 'Chinese Spring' Wheat, 'Imperial' rye, the addition lines of 'Imperial' rye chromosomes into 'Chinese Spring' wheat and the 'Imperial Rye'/'Chinese Spring' wheat amphiploid were obtained from Dr. E. R. Sears, University of

Missouri, USA or Mr. T. E. Miller, Plant Breeding Institute (PBI) Cambridge, UK. Seed of wild species of *Secale* were obtained from Gatersleben, GDH (HR and GRA); Royal Botanic Gardens, Kew; PBI, Cambridge (R); and Instytut Hodowli I. Aklimatyzagi Roslin, Radzikow, Poland. Seed of the wheat cultivars 'Abele' and 'Weique' were obtained from the PBI.

Crosses between inbred lines

Inbred lines derived from a cross between *S. dighoricum* and *S. turkestanicum* (both now regarded as weedy forms of *S. cereale*) were supplied by Dr. G. H. Jones, The University of Birmingham, U.K. Further details of this material are given by Sun and Rees (1964, 1967). The secalin patterns of these lines were determined and crosses made between inbred line 133/6 (as the female parent) and two seed types of inbred line 48/9. Two F_1 plants from each cross were grown together in a glasshouse at Rothamsted. The F_2 seed were analysed for secalin pattern. The data for the two plants of each cross were homogeneous and were combined for analysis.

Extraction and analysis of secalins

Secalins were extracted from *Secale* species as described by Shewry et al. 1978b. The reduced and pyridylethylated fractions were separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a modified Laemmli (1970) system (Forde et al. 1981).

Extraction and analysis of additional lines

Total protein fractions were extracted from the series of 'Imperial Rye'/'Chinese Spring' wheat addition lines and the wheat cultivars 'Abele' and 'Weique' as described by Payne et al. (1980). They were then separated by SDS-PAGE as described by Shewry et al. (1978a). This system gives better resolution of the prolamins of wheat than does the modified Laemmli system.

Statistical analysis

Gene linkage was tested for by χ^2 tests and estimated by the method of maximum likelihood (Bailey 1961). Map distance was calculated using the Kosambi function (Jensen and Jorgensen 1975). Genotype frequencies from different samples were pooled if homogeneous ($P > 0.05$) in contingency χ^2 tests.

Results and discussion

Polymorphism of secalins from S. cereale and related species

SDS-PAGE of secalin fractions from cultivated rye (*S. cereale*) gives a clear separation of the four major groups of secalin polypeptides: the HMW secalins, 75K γ-secalins, ω-secalins and 40K γ-secalins (Fig. 1). Comparison of the polypeptide patterns of secalins from single seeds of one cultivar ('Gazelle') shows considerable variation in the patterns of the first three groups and less extensive variation in the pattern of the 40K γ-secalins. This polymorphism is present because *S. cereale* is an outbreeder, each cultivar being a mixture of genotypes.

The taxonomy of the genus *Secale* is confused, with a total of 16 species being described and recognised by some workers. Roshevitz (1947) recognised 14 species which he classified into three sections, one containing *S. sylvestre* alone and the other two containing *S. cereale, S. montanum* and species related to these. More recent workers have recognised fewer species, suggesting that many of those previously described are weedy forms of *S. cereale* or forms of *S. montanum.* Khush (1962) recognised only five species on cytogenetic grounds, of which *S. sylvestre* was strongly isolated. Other workers recognise seven species (T. E. Miller, personal communication) including *S. anatolicum,* which was included in *S. montanum* by Khush,

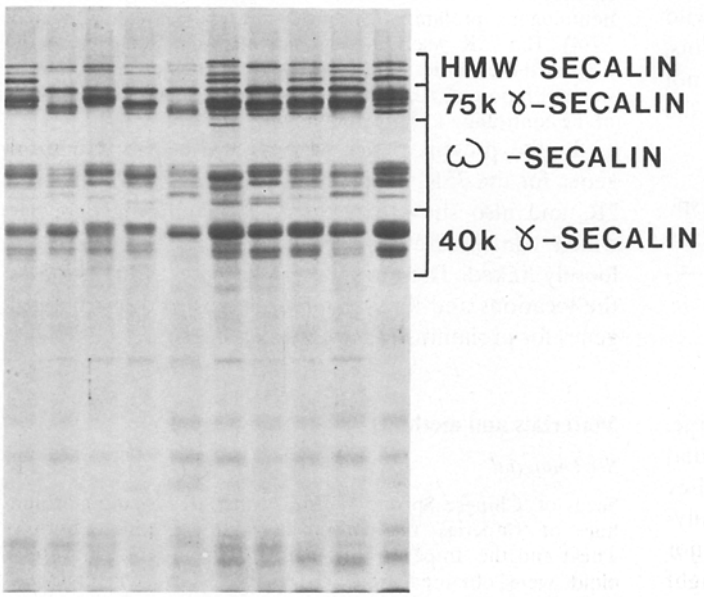

Fig. 1. SDS-PAGE of secalin fractions from single seeds of *S. cereale* cv. 'Gazelle'

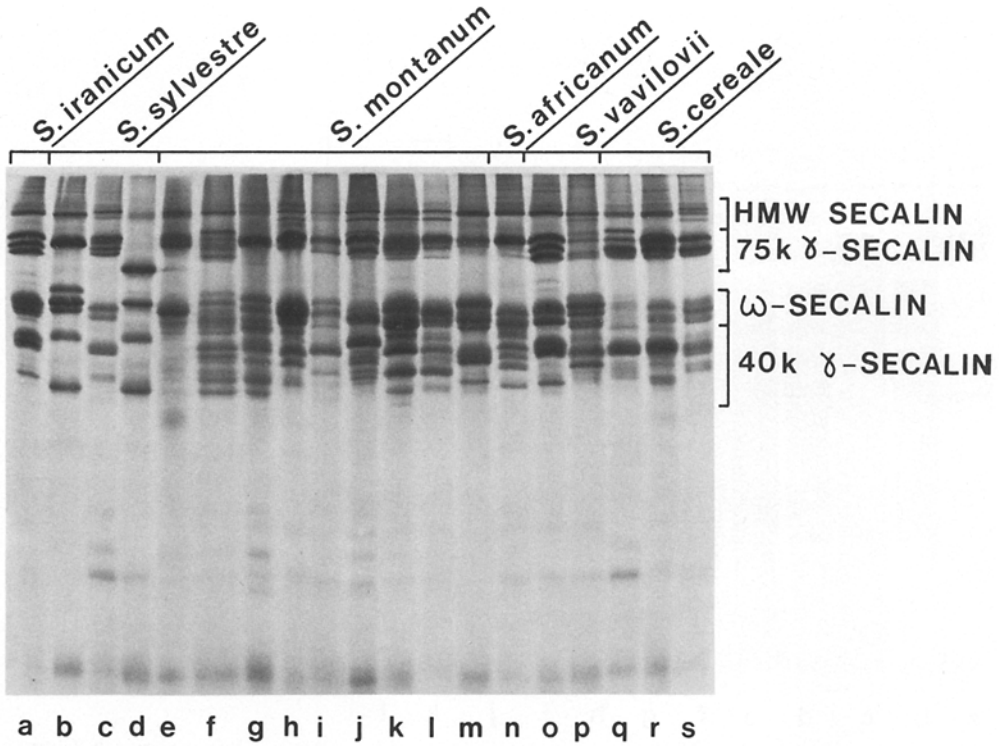

Fig. 2. SDS-PAGE of secalin fractions from single seeds of *Secale* species. *a S. iranicum* (R105); *b S. sylvestre* (R52); *c S. sylvestre* (Radzikow 4028); *d S. sylvestre* (HR 56180); *e S. montanum* (HR. 590/80); *f S. montanum* (Kew 350/2); *g S. montanum* (Radzikow, 4026); *h S. montanum* (R15); *i S. montanum (S. kuprijanovii)* (Radzikow, 4025); *j S. montanum (S. kuprijanovii)* (HR 800/82); *k S. montanum (S. kuprijanovii)* (R100); *l S. montanum (S. dalmaticum)* (R103); *m S. montanum (S. anatolicum)* (R98); *n S. africanum* (R102); *o S. vavilovii* (R109); *p S. vavilovii* (Radzikow 4029); *q S. cereale* cv 'Ashill Pearl'; *r S. cereale* ssp. *multicaulum* (Radzikow, 4024); *s S. cereale (S. segetale)* (Radzikow, 4027). Nomenclatures in brackets are according to the classification of Roshevitz (1947)

and the newly-described *S. iranicum* (Kobylansky 1975).

Figure 2 shows SDS-PAGE separations of secalins from accessions of these species. The patterns of *S. iranicum, S. montanum* (including *S. anatolicum*), *S. africanum* and *S. vavilovii* are similar to those of *S. cereale* with four major groups of polypeptides corresponding in mobility to the HMW secalins, 75K γ-secalins, ω-secalins and 40K γ-secalins. The reasons for the differences in the relative amounts of these groups are not known. The proportions of the homologous groups of prolamins present in wheat and barley are affected by mineral nutrition (Kirkman et al. 1982; Shewry et al. 1983b; Moss et al. 1981), so it would be necessary to grow all the accessions under identical conditions before concluding that the differences shown in Fig. 2 are determined genetically.

In contrast the three accessions of *S. sylvestre* (Fig. 2, b–d) give patterns of a clearly different type, with two major bands in the 40K γ-secalin region and an unusually fast 75K γ-secalin band in one accession (Fig. 2, d). Thus these analyses support the view of Khush (1962) that while *S. sylvestre* is clearly distinct, the other species are closely related, speciation having occurred by geographical isolation and the presence of reciprocal translocations.

The chromosomal locations of the structural genes for secalins

Analysis by SDS-PAGE of the series of addition lines of chromosomes of Imperial rye into 'Chinese Spring' Wheat (Fig. 3) confirmed the presence of two additional HMW bands in the addition line with chromosome 1R (Fig. 3, d). These did not, however, correspond in mobility to the two HMW secalin bands present in the standard stock of 'Imperial' rye (Fig. 3, a), although they were present in the 'Imperial' rye/'Chinese Spring' amphiploid (Fig. 3, c). Thus it is probable that the standard seed of 'Imperial' rye differs in its pattern of HMW secalins from that used in the original cross. Lawrence and Shepherd (1981) also showed that chromosome 1R controlled two HMW secalin bands, which had similar mobilities to those present in the amphiploids. They further showed that these were controlled by genes on the long arm of the chromosome. It was not possible to determine locations of the genes for the ω-secalins and 40K γ-secalins from

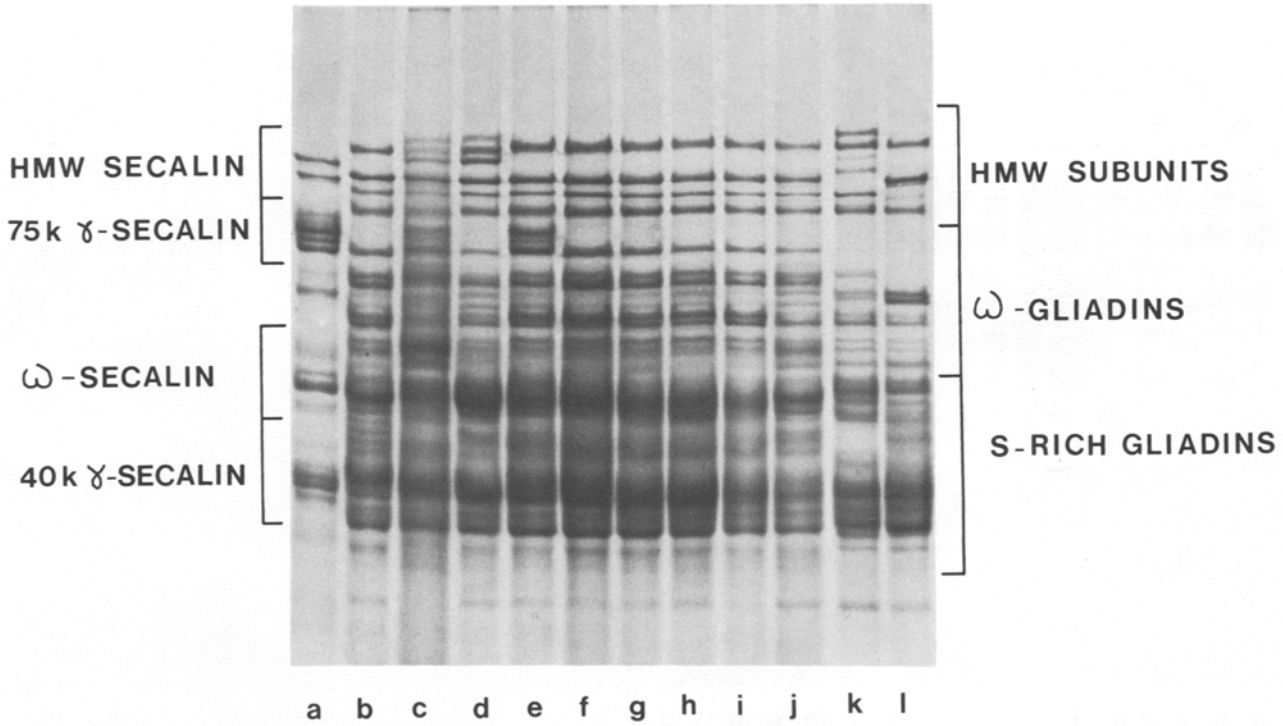

Fig. 3. SDS-PAGE of total protein fractions extracted from wheat, rye and wheat/rye addition lines. *a* Imperial rye; *b* 'Chinese Spring' wheat; *c* Imperial Rye/'Chinese Spring' wheat amphiploid; *d–j* 'Chinese Spring' wheat with chromosomes 1 to 7 of rye added respectively; *k* wheat cv. 'Abele'; *l* wheat cv. 'Weique'. The groups of rye prolamins are indicated on the *left* of the figure and of wheat prolamins on the *right*

the SDS-PAGE separations, but Shepherd and co-workers (Shepherd 1968, 1973; Shepherd and Jennings 1971; Lawrence and Shepherd 1981) have shown that these are located on chromosome 1R, with at least some present on the short arm.

The 75K γ-secalins were not present in the addition line with chromosome 1R but were clearly observed in the chromosome 2R addition line (Fig. 3, e). Again the precise band pattern present in this region was identical to that in the amphiploid (Fig. 3, c) rather than to that in the standard seed of 'Imperial' rye (Fig. 3, a). Lawrence and Shepherd (1981) also showed the presence of an unidentified band with a similar mobility to the 75K γ-secalins in the chromosome 2R addition line. To confirm that the genes for the 75K γ-secalins were not located on chromosome 1R we also analysed two varieties of wheat, one with chromosome 1R substituted for chromosome 1B (Weique Fig. 3, l) and one with the short arm of chromosome 1R substituted for that of chromosome 1B (Abele Fig. 3, k). The 75K γ-secalins were not present in either line.

Linkage relationships of the structural genes for secalins

Seeds of several inbred lines from a cross between two weedy forms of *S. cereale* (previously described as *S. turkestanicum* and *S. dighoricum*) were screened by SDS-PAGE. Three seed types had polypeptide patterns which made them suitable parents for crosses. Two were seed types of inbred line 48 (called – 48A and 48B) and the third was line 113. These did not segregate for secalin pattern when selfed, and were therefore assumed to be homozygous. Three bands, numbred 1–3 in Fig. 4, a–c, were present in only one or two of the lines. These were identified as a HMW secalin, a 75K γ-secalin and a ω-secalin respectively (Table 1). Chemical evidence for these identifications is as follows:

1. Bands 1 and 2 were not resolved by SDS-PAGE unless extracted and/or separated in the presence of a reducing agent. This indicates that they are present in

Table 1. The identifies of bands 1–3 and their presence in the parents of crosses 1 and 2

Band no. Band identity	1 HMW secalin	2 75k γ-secalin	3 ω-secalin
Inbred line 133	+	–	–
Inbred line 48 type A	–	+	+
Inbred line 48 type B	–	–	+

the seed as aggregates stabilised by disulphide bonds, which is characteristic of HMW secalins and 75K γ-secalins (Shewry et al. 1983c; Field et al. 1983). Both bands were cleaved by cyanogen bromide. This indicates the presence of methionine in the proteins, and is consistent with the identification (Shewry et al. 1982; Field et al. 1982).

2. Band 3 was clearly resolved without reduction and was not apparently cleaved by cyanogen bromide. This is consistent with its identification as an ω-secalin, a group of prolamins characterized by an absence of cysteine and the presence of only a trace of methionine (Charbonnier et al. 1981; Kasarda et al. 1983). Although the M_r of this component was considerably higher than that of the other ω-secalins, this is not unusual for this type of prolamin. The homologous ω-gliadins and C hordeins of wheat and barley respectively aso vary widely in their M_rs (Faulks et al. 1981; Kasarda et al. 1983).

Band 1 was present only in inbred line 133 (Fig. 4, c). Band 3 was present in both seed types of line 48, and band 2 in type A only (Fig. 4, a, b). The presence of bands 1–3 in the three seed types is summarised in Table 1. The seed types also showed other differences in the patterns of the 75K γ-secalins, ω-secalins and the 40K γ-secalins (Fig. 4, a–c).

Both seed types of line 48 were crossed with 133. Examination of secalin fractions from single F_2 grain (Fig. 4, d–u) showed a range of polypeptide patterns. In some seed the patterns of one or more of the groups of secalins were identical to those of one of the parent lines, for example the fraction separated in Fig. 4, n is identical to 48 in the HMW secalins and to 133 in the 75K γ-secalins, ω-secalins and 40K γ-secalins. This is presumed to be homozygous for the controlling genes. In other seed the patterns of both parents were present, and it was sometimes possible to distinguish two types differing in the relative intensities of these, for example of band 1 in Fig. 4, h and t. These are assumed to be two classes of heterozygote differing in the dosages in the triploid endosperm of alleles derived from the male and female parents. These results indicate that secalins are controlled by co-dominant alleles, as has been described previously for hordein of barley (Shewry et al. 1978b, 1983) and gliadins and glutenins of wheat (Payne et al. 1982, 1984).

When scoring the F_2 grain it was not always possible to distinguish between the three phenotypic classes with bands 1, 2 or 3 present. We therefore scored the seed only for absence or presence of these bands. Thus the alleles conferring band presence were treated as dominant, and a segregation ratio in the F_2 generation of 9 : 3 : 3 : 1 should have been observed if the structural genes for two bands were not linked.

The numbers of F_2 plants in the various phenotypic classes are given in Table 2. This also gives the linkage

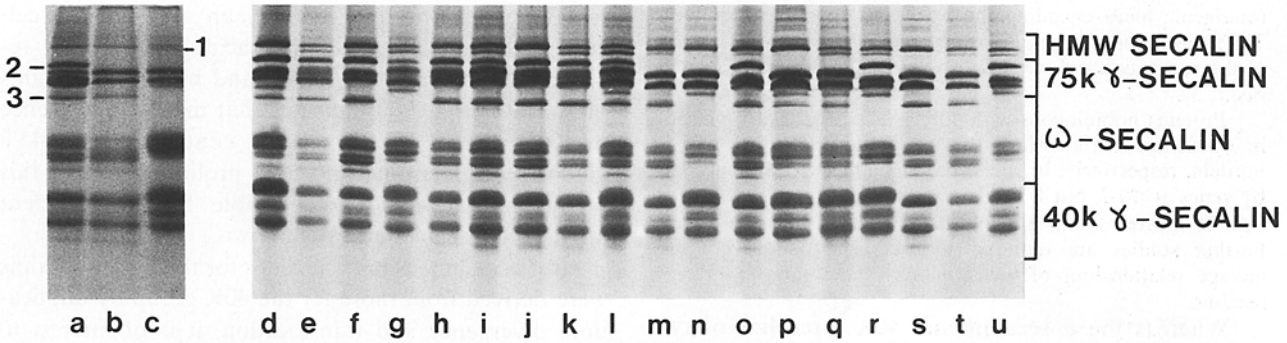

Fig. 4. SDS-PAGE of secalins from single seeds of the parents of crosses *1* and *2* (*a–c*) and F_2 seed from cross *2* (*d–u*). *a* inbred line 48 type A; *b* inbred line 48 type B; *c* inbred line 133; *d–u* F_2 seed from cross *2* (133×48 type A)

Table 2. Phenotypic classes, linkage χ^2, probability of independence, recombination percentages and map distances for the bands segregating in crosses 1 (133×48 type B) and 2 (133×48 type A)

Cross	Bands	Total no. of progeny	Nos. of progeny in phenotypic classes				Linkage χ^2 df = 1	Probability of independence	Recombination percentage (± SE)	Map distance in cM (± SE)
			++	+−	−+	−−				
1	1 & 3	150	83	35	25	7	0.91	> 0.2	43.8±6.64	
2	1 & 3	316	167	67	69	13	5.60	< 0.05	39.4±4.62	
1+2 pooled	1 & 3	466	250	102	94	20	6.21	< 0.05	40.8±3.76	57.4±11.30
2	1 & 2	316	176	58	58	24	0.64	> 0.3	53.2±3.93	
2	2 & 3	316	177	57	59	23	0.43	> 0.5	47.3±4.07	

χ^2 values, the recombination percentages and, where the χ^2 value indicates linkage, the map distance.

The results of cross 2 showed no significant linkage between bands 1 and 2 or bands 2 and 3. This is consistent with the results obtained with the addition lines, which showed that the structural genes for band 2 were located on chromosome 2R and those for bands 1 and 3 on chromosome 1R. In contrast the combined results of both crosses showed significant linkage between bands 1 and 3. The recombination percentage was 40.8 ± 3.76 and the map distance 57.4 ± 11.30 cM.

It was not possible to score all the bands segregating in the 75K γ-secalins, ω-secalins and 40K γ-secalins in all F_2 seed. However, where they could be scored they indicated that band 2 was linked to the other 75K γ-secalins, and band 3 to the other ω-secalins and to the 40K γ-secalins.

General discussion

The results reported here show that secalins are governed by co-dominant alleles located at at least three loci on two different chromosomes.

The genes encoding ω-secalins are probably located on the short arm of chromosome 1R (Lawrence and Shepherd 1981). Also, loci encoding the homologous prolamins of wheat (ω-gliadins) and barley (C hordein) are located distally on the short arms of the homoeologous chromosomes 1A, 1B and 1D of wheat and chromosome 5 of barley. These have been designated *Gli-1* and *Hor 1* respectively (Payne et al. 1982; Doll and Brown 1979) and each appears to be complex multigenic locus encoding the whole group of polypeptides. The locus mapped here also appears to encode most, if not all, of the ω-secalins and we propose that it should be designated *Sec 1*.

Proteins homologous with 40K γ-secalins are also present in wheat and barley where they are called γ-gliadins and B hordein, respectively. In wheat the γ-gliadins are also encoded by genes at *Gli-1*, but B hordein is encoded by a second locus (*Hor 2*) located about 8 cM distally to *Hor 1* (Jensen 1983). Further studies are required to establish conclusively the linkage relationships of the structural genes for the 40K γ-secalins.

Whereas the ω-secalins and 40K γ-secalins of rye are present in the seed as monomers associated by non-covalent interactions, the HMW and 75K γ-secalins are present in aggregates stabilised by disulphide bonds (Shewry et al. 1983c; Field et al. 1983). In wheat the monomeric components are called gliadins and the aggregates glutenins, although both types of polypeptide are soluble in aqueous alcohols after reduction and can therefore be defined as prolamins. The HMW secalins are homologous with the HMW subunits of wheat glutenin and with D hordein of barley. Lawrence and Shepherd (1981) showed that the structural genes for these were located on the long arms of chromosomes 1R of rye, 1A, 1B and 1D of wheat and 5 of barley. The controlling loci in wheat (*Glu-1*) and barley (*Hor 3*) have since been accurately mapped and shown to be close to the centromeres (about 9 cm), with map distances from *Gli-1* and *Hor 1* of about 65 cM (Payne et al. 1982; Shewry et al. 1983b). This is similar to the map distance between *Sec 1* and the locus for HMW secalins, which we propose should be designated *Sec 3*.

Lawrence and Shepherd (1981) also reported that a major endosperm protein was encoded by genes on chromosome 2R, although this was before the 75K γ-secalins had been purified and characterized (Shewry et al. 1982). Although these genes have not been mapped on the chromosome, we propose that they should be provisionally designated *Sec 2*. This is not homologous with *Hor 2* of barley which encodes B hordein or *Gli-2* of wheat which is located on the group 6 chromosomes (Payne et al. 1982) and encodes a group of gliadins which have no close homologues in barley or rye (Shewry et al. 1984).

The two groups of γ-secalins have related N-terminal amino acid sequences (Shewry et al. 1982), indicating a structural relationship. They differ, however, in their molecular weights, amino acid compositions and aggregation properties. Since homologues of the 75K γ-secalins are not apparently present in wheat and barley they can be regarded as a derived form. The 75K γ-secalins have increased proportions of glutamate + glutamine (40 mole%) and proline (23.5 mole%) compared to the 40K group (35 and 18 mole%, respectively). This suggests that they may be derived from 40K γ-secalins by the insertion of a sequence rich in glutamine and proline. In fact, an approximate calculation based on the amino acid compositions reported by Shewry et al. (1982) and molecular weights of 75,000 and 40,000 indicates that the extra sequence present in the 75K γ-secalins contains about 45% glutamate + glutamine and 30% proline. Whether this or other changes are responsible for the different aggregation behaviour is not known.

If it is assumed that the genes for the 75K γ-secalins were derived from those for the 40K group by duplication, divergence and translocation, it is of interest to consider the sequence and timing of these events. Since 75K γ-secalins appear to be present in all species of *Secale* (Fig. 1), the duplication and divergence can be assumed to predate the speciation of the genus. Translocations are considered to be an important factor in the speciation of *Secale* (Khush 1962), although they have not been reported between chromosomes 1R and 2R. However, an event of this type must have occurred prior to the separation of *S. cereale*, if not earlier in the evolution of the genus. To provide further information on these events we are studying the locations of the structural genes for secalins in other species by using in situ hybridization of radioactively-labelled cDNAs related to 40K and 75K γ-secalins to preparations of chromosomes.

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Note added in proof: N.K. Singh and K.W. Shepherd have shown that the structural genes for the HMW secalins (which they designate *Glu-R1*) are located 4.65 ± 1.04 cM from the centromere on the long arm of chromosome 1R (manuscript submitted). This is in agreement with our results.