Genetic Control of Rye Endosperm Proteins

It has been shown that the genetic control of many of the electrophoretically slow-moving protein bands (gliadins) in the endosperm of hexaploid Chinese Spring wheat is associated with the chromosomes of homoeologous groups 1 and 6[1]. Hence it may be inferred that at least 2 chromosomes were associated with the equivalent proteins of the ancestral diploid species from which the 3 genomes of wheat are believed to be derived[2]. Since the genome of diploid rye, *Secale cereale*, is thought to be derived from this same diploid progenitor[3], it was expected that 2 chromosomes would also be concerned with the slow-moving endosperm proteins in rye. In an analysis of wheat-rye addition lines, involving single chromosome pairs of King II rye added to Holdfast wheat[4,5], only one rye chromosome (V) could be associated with these proteins[1]. However, at least 2 of the rye bands had similar mobilities to wheat bands and they could not be clearly distinguished in the amphiploid or the addition lines. In the present study, more critical tests have been made to determine whether the gene(s) controlling these 2 rye bands are located on a chromosome other than V and thus to check whether the chromosomal control of endosperm proteins in rye is similar to that in wheat.

Improved resolution of rye bands was obtained by using 0.01 *M* sodium pyrophosphate buffer (pH 7.4)[6] instead of 2 *M* urea as the protein extractant. With 2 *M* urea the slow-moving proteins of rye and wheat appeared to be extracted in approximately equal amounts as judged by the staining intensity of the bands in starch gels[1]. However, when pyrophosphate buffer was used as the solvent the slow-moving bands of Holdfast wheat did not stain or at most stained faintly, whereas the equivalent bands of King II rye were observed to stain quite strongly (see Figure 1). It follows that if this difference in solubility of wheat and rye proteins also applies when they are mixed together, as in the amphiploid and relevant addition lines, it would be possible to differentiate rye bands from wheat bands even if they have the same mobility in gels.

Thus the material previously compared using a 2 *M* urea extract from single grains[1] has been compared again using pyrophosphate buffer as the protein solvent. This material consists of Holdfast wheat ($2n = 42$), King II rye ($2n = 14$), the Holdfast + King II amphiploid ($2n = 56$), and seven separate wheat-rye addition lines ($2n = 44$). The conditions of electrophoresis were the same as before except the running time was reduced from 7.5 to 2.5 h in some tests to show the fast-moving proteins as well as the slow-moving fraction (e.g. Figure 1).

The gel pattern of the slow-moving proteins in pyrophosphate extracts from the amphiploid usually did not possess any well defined wheat bands, but was comparable with the pattern of the rye parent, except for an additional faint band (No. 4, Figure 1), and included those rye bands (No. 5, 6 and 7) not resolved in 2 *M* urea extracts. Since the pattern of the chromosome V addition lines (complete and short-arm telo) appeared to be identical with that of the amphiploid, it is concluded that all of the slow-moving proteins of rye are controlled by gene(s) on the short arm of rye chromosome V. Rye bands were not detected in any of the pyrophosphate extracts from the other six addition lines, thus supporting this conclusion.

Following the success of the above analysis, similar tests were made on addition lines involving Imperial rye and Chinese Spring wheat[7], but the comparisons were restricted to the wheat and rye parents, the amphiploid, and the addition line (E) known to possess rye endosperm proteins[1].

The gel patterns obtained (Figure 2) were more difficult to interpret than those described above, because of a greater interference from wheat bands and because the pattern of rye bands from the amphiploid did not agree closely with that from the Imperial rye control.

[1] K. W. SHEPHERD, Proc. 3rd Int. Wheat Genet. Symp. (Australian Academy of Science, Canberra 1968), p. 86.
[2] E. R. SEARS and M. OKAMOTO, Proc. Int. Genet. Symp. Supl. vol. Cytologia, 1957, p. 332.
[3] E. R. SEARS, Proc. 3rd Int. Wheat Genet. Symp. (Australian Academy of Science, Canberra 1968), p. 53.
[4] R. RILEY and V. CHAPMAN, Heredity *12*, 301 (1958).
[5] R. RILEY, Heredity *14*, 89 (1960).
[6] J. H. COATES and D. H. SIMMONDS, Cereal Chem. *38*, 256 (1961).
[7] E. R. SEARS, unpublished.

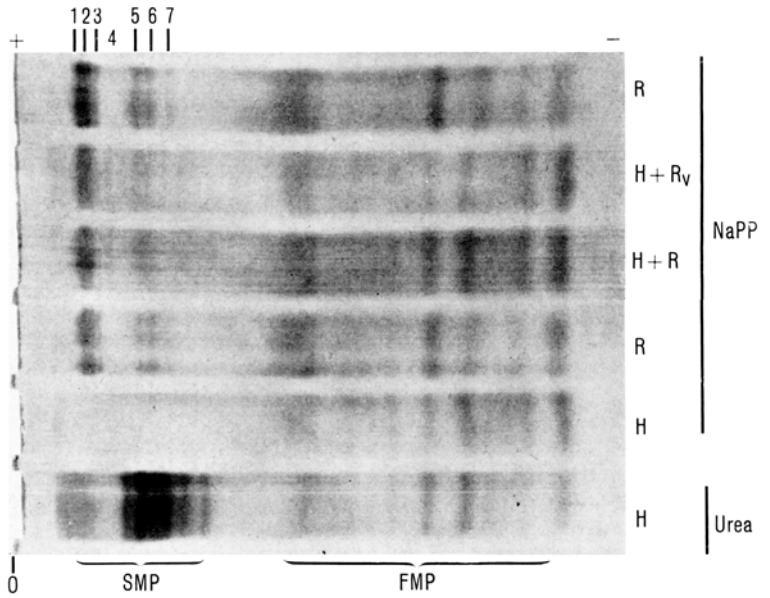

Fig. 1. Starch-gel electrophoretic patterns of slow-moving (SMP),and fast-moving (FMP) endosperm proteins from Holdfast wheat (H), King II rye (R), the amphiploid (H + R) and rye chromosome V disomic addition line ($H + R_v$) extracted in 0.01 *M* sodium pyrophosphate (NaPP) or 2 *M* urea. Electrophoresis for 2.5 h at 11.5 V/cm.

Besides lacking 2 of the bands present in Imperial rye (No. 4 and 7), the amphiploid possessed a band (No. 5) not present in either parent cultivar. These differences in pattern could be due either to an interaction between wheat and rye proteins when in a common cytoplasm or simply to the Imperial rye used as a control in the present study differing in genotype from the rye plant used in constructing the amphiploid. The latter explanation is favoured[8] because it was found that the pattern of rye bands in F_1 seeds derived from crossing Chinese Spring with the Imperial rye control resembled the pattern of the rye parent rather than that of Sears' amphiploid. However, irrespective of the cause of the pattern difference, it is clear that the gene(s) controlling all of the rye bands expressed in the amphiploid are located on rye chromosome E, since the pattern of the chromosome E addition line was identical with that of the amphiploid (Figure 2).

To summarize, it is evident that the gene(s) controlling all of the slow-moving proteins of diploid King II rye, and probably Imperial rye also, are located on one particular rye chromosome, whereas in hexaploid Chinese Spring wheat the genetic control of these proteins is associated with at least 2 chromosomes of each genome. It is concluded that the genome of rye must have evolved along a separate pathway from that of the 3 wheat genomes, since it is widely accepted that a change in the number of loci controlling a protein phenotype is a reliable index of evolutionary divergence[9,10]. On the other hand, evidence from studies of chromosome homoeology indicates that the rye and wheat genomes have most likely been derived from a common ancestral genome[3]. To account for these separate findings it is postulated that the gene(s) controlling the slow-moving proteins in the original diploid ancestor of the Triticinae were located on only 1 of the 7 chromosomes, and that rye was derived from this species. Further it is suggested that a descendant of this ancestral diploid acquired two-chromosome control of these proteins, possibly by a process of gene duplication and chromosome translocation, analogous to that suggested for the origin of the α, β, γ and δ polypeptide chains of haemoglobin[9], and that the wheat genomes have come from this derived species.

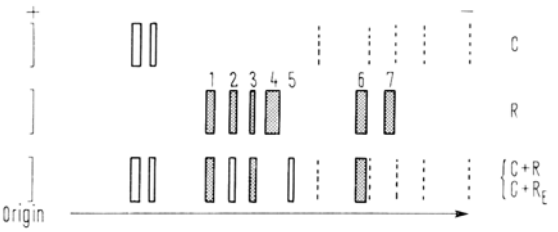

Fig. 2. Diagram of starch-gel electrophoretic patterns of slow-moving proteins extracted in 0.01 *M* sodium pyrophosphate from Chinese Spring wheat (C), Imperial rye (R), the amphiploid (C + R) and rye chromosome E addition line (C + R_E). Electrophoresis for 7.5 h at 11.5 V/cm. (Shaded rectangles, strong bands; clear rectangles, weak bands; dotted lines, faint bands often difficult to detect.)

If this model is correct, the chromosome segments controlling the slow-moving proteins in wheat and rye would be related by descent and, consequently, the respective proteins would be expected to have similar amino acid sequences and similar chemical properties. However, the different behaviour of these proteins during pyrophosphate extraction seemed to be inconsistent with this expectation, and a quantitative study of their solubility in pyrophosphate buffer was made to resolve this anomaly. It was found that although the slow-moving proteins of rye are more soluble in this buffer than those of wheat, the difference is small and probably does not represent a fundamental difference in their chemical properties[11].

The proposed pathway for the evolution of wheat and rye genomes obviously needs further testing and additional data are expected to come from determining the number of chromosomes controlling endosperm proteins in other genomes within the Triticinae or, ideally, comparing the amino acid sequences of the proteins involved[12].

Résumé. En comparant les diagrammes d'électrophorèse en gels d'amidon de gliadine obtenus à partir d'un blé hexaploïde, d'un seigle diploïde et de blé + seigle amphiploïdes avec les lignes d'additionnement des chromosomes blé-seigle on constata qu'un chromosome de seigle contrôle toutes les gliadines du seigle.

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[8] A similar explanation may account for the faint band (No. 4, Figure 1) which was observed consistently in the pyrophosphate extracts from Holdfast + King II amphiploid and the chromosome V addition lines, but which was absent from the wheat and rye controls.

[9] V. M. Ingram, Nature, Lond. *189*, 704 (1961).

[10] S. Ohno, U. Wolf and N. B. Atkin, Hereditas *59*, 169 (1968).

[11] K. W. Shepherd and A. C. Jennings, unpublished.

[12] The authors would like to thank Miss B. M. Simons for technical assistance. Financial support from the Wheat Industry Research Council is gratefully acknowledged.

Modes of Variation in Alcohol Dehydrogenase in *Drosophila melanogaster*

It has previously been shown[1-3] that crude enzyme extracts of the two naturally occurring electrophoretic variants of *Drosophila melanogaster* alcohol dehydrogenase can differ in their thermolabile properties and specific activities. We have compared Adh alleles extracted from sternopleural selection lines maintained by Prof. A. Robertson, from a cage population polymorphic for Adh alleles initiated by Prof. J. A. Beardmore, and also Adh alleles originally isolated by Prof. E. H. Grell and subsequently put on a common genetic background.

Homogenates of 20 third instar larvae in 1 ml 0.05 *M* orthophosphate buffer were centrifuged at 30,000 *g* for 20 min. The supernatants were assayed for alcohol dehydrogenase activity in a Perkin-Elmer 124 spectrophotometer at 25 °C by timing the change in 0.1. O.D. units at 340 nm associated with the reduction of nicotinamide adenine dinucleotide (NAD) using isopropanol as