Wheat Storage Proteins: Their Genetics and Their Potential for Manipulation by Plant Breeding [and Discussion]

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Wheat storage proteins: their genetics and their potential for manipulation by plant breeding

By P. I. Payne, Linda M. Holt, Elizabeth A. Jackson and C. N. Law

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[Plate 1]

The genes that code for endosperm storage proteins occur at nine complex loci on six different chromosomes. *Glu-A1*, *Glu-B1* and *Glu-D1* contain the genes for high molecular mass subunits of glutenin and are close to the centromere on the long arms of chromosomes 1A, 1B and 1D respectively. On the short arms of the same chromosomes, but distant from the centromere, are *Gli-A1*, *Gli-B1* and *Gli-D1*. Each of these loci carry three major gene families coding for ω-gliadins, γ-gliadins and low molecular mass glutenin subunits. The remaining loci, *Gli-A2*, *Gli-B2* and *Gli-D2* occur near the ends of the short arms of chromosomes 6A, 6B and 6D respectively and each code for α- and β-gliadins. Recombination of genes within a locus is very rare and has so far been detected only at *Glu-B1*, at the rate of about one recombinant in 1000 progeny. Each locus displays allelic variation and this is responsible for differences among varieties in protein quality for making bread. The protein variants that are associated with good quality are being identified, firstly by analysing segregating populations and secondly from the development of near-isogenic lines. Current, incomplete, information on the relative qualities of different alleles at each locus indicates the following order of importance: *Glu-1* > *Gli-1* > *Gli-2*. Landraces of primitive agriculture are being screened for novel proteins. The genes for some of them are being incorporated into the genomes of commercial wheats.

Introduction

The protein content of cereal grains is low and for wheat is normally between 9% and 16% of the dry weight. Nevertheless, world production of wheat grain protein is vast, about 40×10^6 t on average (Harlan & Starks 1980), compared with only 23×10^6 t protein from the protein-rich soybean crop. As well as being of great importance nutritionally to many peoples of the world, wheat grain protein plays a fundamental part in food processing, for instance, in bread manufacture, biscuits, breakfast cereals and pasta products (Payne & Rhodes 1982). On the whole, western European wheats are not ideal for making bread, partly because of their low protein content (9–11% of their dry weight) but also because of their poor protein quality. In the United Kingdom, good quality bread wheats are traditionally imported from North America at high cost to improve the quality of bread grists. Accordingly, a major priority at the Plant Breeding Institute, Cambridge, is to produce high-yielding bread wheats with improved bread-making quality. To understand the meaning of protein quality for bread-making, the proteins of the endosperm have been characterized by gel electrophoresis and their controlling genes have been subjected to genetical analysis. A more detailed account of the biochemistry of the endosperm storage proteins and their relationship to those of other cereals is given by P. R. Shewry, B. J. Miflin and D. D. Kasarda (this symposium).

[85]

Genetic constitution of bread wheat

Bread wheat (*Triticum aestivum*) is hexaploid, consisting of three different diploid genomes termed AA, BB and DD. The three genomes are known to be closely related and the original genome donors were diploid species of *Triticum and Aegilops* (Riley 1965). Each genome consists of seven pairs of chromosomes giving a total of 21 pairs in bread-wheat nuclei (table 1). Each chromosome pair is given a number followed by a letter, the letter referring to the genome from

Table 1. The chromosomes of bread wheat

possible donor	A genome *Triticum urartu*	B genome unknown	D genome *Aegilops squarrosa*
chromosome			
1	<u>1A</u>	<u>1B</u>	<u>1D</u>
2	2A	2B	2D
3	3A	3B	3D
4	4A	4B	4D
5	5A	5B	5D
6	<u>6A</u>	<u>6B</u>	<u>6D</u>
7	<u>7A</u>	7B	7D

The chromosomes that carry the storage protein genes are underlined.

which the pair was inherited. Chromosomes from different genomes that are genetically similar (or homoeologous) to each other are given the same number, for instance chromosomes 1A, 1B and 1D. As will be seen, the A, B and D genomes have sets of storage proteins that are electrophoretically distinguishable from each other, because of mutation of their structural genes since the divergence of the common ancestor of the A, B and D genome donors. It follows therefore that bread wheat will have many more storage protein components than related diploid species such as barley, *Hordeum vulgare*.

As the chromosomes in hexaploid wheat are effectively triplicated, partial or whole chromosome deletions are usually not lethal, for essential genes on eliminated chromosomes will be present on their homoeologues. These aneuploid lines have been systematically developed, initially by Sears (1954) in the variety Chinese Spring, and they have been invaluable in the genetic analysis of storage proteins, particularly in the study of gene location and gene linkage. Relevant to the work described here are two types: nullisomic(N)-tetrasomic(T) lines and ditelocentric (DT) lines, all derived from the variety Chinese Spring (CS). As examples, the line CS N1A T1B lacks the pair of 1A chromosomes but has double the normal complement of 1B chromosomes to maintain the chromosome number at 42; the line CS DT 1AL lacks the short (S) arm of chromosome 1A but possesses the corresponding long (L) arm. Thus, a protein that is present in Chinese Spring, absent in CS N1A T1B and present in CS DT 1AL must have its controlling genes on the long arm of chromosome 1A.

Intervarietal chromosome substitution lines are also of prime importance in the study of endosperm protein genetics, particularly allelic variation and gene linkage. These are lines in which individual pairs of homoeologous chromosomes of a recipient variety have been replaced by their homoeologues from a donor variety and many have been developed at the Plant Breeding Institute (Law & Worland 1973; Law *et al.* 1981). As an example, Koga II (Bersée 1A), has the same genetic constitution as Koga II except its 1A chromosomes have been replaced by those from Bersée.

Definitions of storage proteins

Classification of the storage proteins in wheat is fraught with difficulties and no one system satisfies all researchers. Usually two protein groups are recognized in wheat, glutenin and gliadin, though their actual compositions will vary, according to how they are prepared. The gliadin group is now most usually defined as comprising those proteins that are soluble in 70 % aqueous ethanol at room temperature and the glutenin group as those that are insoluble but which dissolve in dissociating media: mixtures of dilute acid, urea and ionic detergents. For reasons given elsewhere (Jackson *et al.* 1983) we prefer to classify these two protein groups according to their state of aggregation in dissociating media rather than their solubility. Gliadin occurs as a complex mixture of single polypeptides whereas glutenin consists of subunits that are aggregated together, mainly by disulphide bonds. For this classification, gliadin and glutenin are simply and effectively separated by gel filtration. The net effect of the change in definition is to transfer about 10 % of the gliadin fraction, the component termed variously as high molecular mass gliadin (Beckwith *et al.* 1966), low molecular mass glutenin (Nielsen *et al.* 1968) and glutenin III (Graveland *et al.* 1982) to glutenin. By two dimensional electrophoresis, their constituent subunits were shown to be identical to the major subunit group of glutenin and quite distinct from the bulk of the major gliadin polypeptides (Jackson *et al.* 1983).

Both glutenin and gliadin are deposited in storage proteins in the developing grain (Miflin *et al.* 1980) and both are considered to be prolamins (the aqueous alcohol soluble proteins of Osborne 1907), in accord with the work of Shewry *et al.* (this symposium). The two protein groups have very different biophysical properties: glutenin imparts elasticity to a bread dough whereas gliadin is viscous and gives extensibility. When glutenin is treated with a reducing agent such as 2-mercaptoethanol, it loses its elastic properties and dissociates into several subunits that are here classified into two groups, low molecular mass (l.m.m.) subunits and high molecular mass (h.m.m.) subunits†. The storage proteins of wheat flour typically consists of approximately 50 % gliadin, 10 % h.m.m. glutenin subunits and 40 % l.m.m. glutenin subunits.

Chromosomal location of the storage protein genes

The first major contribution to gene location was published by Shepherd (1968), who studied the gliadin proteins of nulli–tetra and ditelocentric lines of Chinese Spring by starch gel electrophoresis at pH 3.2 using aluminium lactate buffer. On the basis of presence and absence of electrophoretic bands in different stocks, genes for nine gliadins were shown to be located on chromosomes 1AS, 1BS, 1DS, 6AS and 6DS. The remaining electrophoretic bands did not vary in any of the genetic lines, presumably because they consisted of mixtures of gliadins whose genes were located on different chromosomes.

Wrigley & Shepherd (1973) improved greatly the resolution of gliadins by the application of two dimensional electrophoresis, isoelectric focusing (i.e.f.) in the first dimension and starch gel electrophoresis at pH 3.2 in the second. They showed that all the gliadin protein genes were located on either chromosomes 1A, 1B, 1D, 6A, 6B or 6D. These results were confirmed recently by Payne *et al.* (1982a), who used a different two dimensional system, polyacrylamide gel electrophoresis at pH 3.1 (a.p.a.g.e.) followed by a sodium dodecyl sulphate (SDS) p.a.g.e.

† These fractions correspond to the HMW and LMW fractions described by the author in previous papers.

in the second. Both groups showed that all the ω-gliadins, most of the γ-gliadins and a few of the β-gliadins are controlled by the group 1 chromosomes whereas all the α-gliadins, most of the β-gliadins and a few γ-gliadins are coded on the group 6 chromosomes. In addition, Payne *et al.* (1982*a*) showed that all the genes occurred on the short arms of the homoeologous group 1 and group 6 chromosomes.

The advent of SDS p.a.g.e., which principally fractionates by molecular mass, enabled the genes controlling the h.m.m. subunits of glutenin to be determined. By using similar genetic stocks to those used for gliadin analysis, Bietz *et al.* (1975) showed that the genes that control these glutenin subunits are on the long arms of chromosomes 1 B and 1 D. These results have since been confirmed (Lawrence & Shepherd 1980; Payne *et al.* 1980). Lawrence & Shepherd (1981*a*) also showed that chromosome 1AL also carries genes for these subunits.

The location of the genes that control the l.m.m. subunits of glutenin has proved to be a much more difficult task. These proteins remain in their aggregated state as glutenin during the gliadin extraction procedure for a.p.a.g.e. Consequently only a small proportion of the subunits dissolve and those that do either remain at the origin of the gel or produce background streaking (Charbonnier 1973). The l.m.m. subunits are extracted by SDS buffer but on reduction and SDS p.a.g.e. they have very similar mobilities, both to each other and to several of the γ-gliadins. To overcome this, Jackson *et al.* (1983) fractionated total endosperm protein by the two different, two dimensional systems of O'Farrell (1975) and O'Farrell *et al.* (1977) that is, i.e.f., followed by SDS p.a.g.e. and non-equilibrium pH-gradient electrophoresis (n.e.p.h.g.e.) followed by SDS p.a.g.e. (abbreviated to n.e.p.h.g.e./i.e.f. × SDS p.a.g.e.). The resolution achieved for Chinese Spring proteins by these combined techniques is shown in plate 1, figure 1. The l.m.m. subunits of glutenin split into a major group of basic proteins and two minor groups, one of which contains acidic proteins and the other, near-neutral proteins. The latter were obscured by the more prominent gliadins and their gene locations could not be determined. However, the l.m.m. glutenin subunits of the other groups were shown to be determined by genes on the short arms of chromosomes 1 A, 1 B and 1 D, like the genes for the ω- and γ-gliadins.

Also revealed in figure 1 are two other groups of proteins. The globulins form three streaks in this system. Their intracellular location in the developing endosperm and their function is not known. The slowest moving streak in the second dimension is known to consist of a protein coded by genes on chromosome 4 D (Brown & Flavell 1981). The function of the other group in figure 1 is also not known, though they appear not to be storage proteins and their controlling genes do not occur on the group 1 or group 6 chromosomes.

The chromosomal location of the endosperm storage protein genes is summarized in table 2.

Variation and linkage

When different varieties of wheat to Chinese Spring are studied by electrophoresis, quite different banding (or spot) patterns are often obtained for all three storage protein groups. Indeed, protein variation is made use of commercially to identify varieties and varietal mixtures by one-dimensional gliadin electrophoresis (J. R. S. Ellis, this symposium).

The simplest way of interpreting this genetical variation is to analyse series of intervarietal chromosome substitution lines: variation can then be ascribed to individual chromosomes. Figure 2 shows the gliadin protein map of Chinese Spring, obtained by a.p.a.g.e. × SDS p.a.g.e.
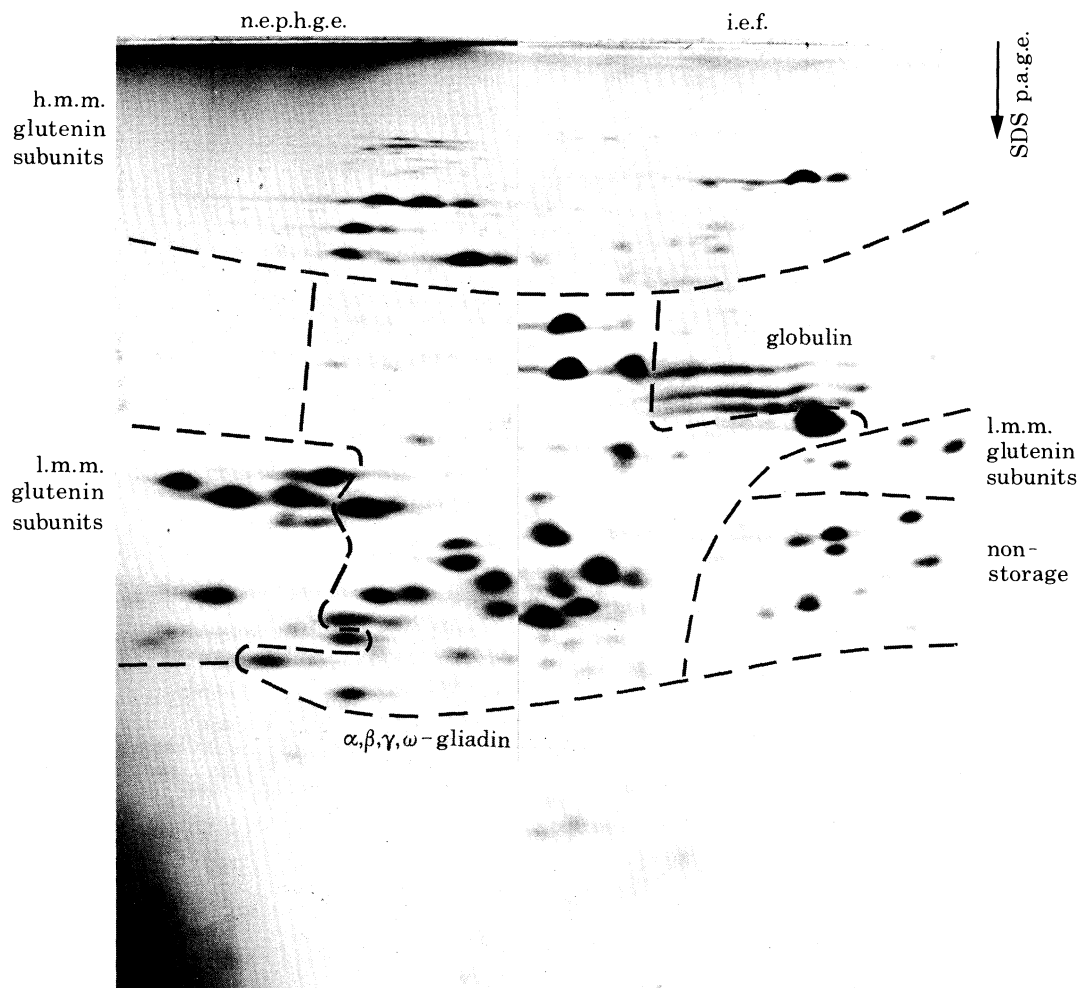
FIGURE 1. Fractionation of the total endosperm proteins of the variety Chinese Spring by two different two dimensional electrophoresis systems: n.e.p.h.g.e. × SDS p.a.g.e. and i.e.f. × SDS p.a.g.e. The distinction between glutenin subunits and gliadins was determined by gel filtration chromatography and the identification of globulin proteins was by their solubility in 0.5 M sodium chloride.

From a similar analysis of six different substitution lines of 1 A chromosomes into Chinese Spring, the different map positions of chromosome 1 A ω- and γ-gliadins have been drawn as open circles.

The variation in protein patterns between varieties has been used to determine whether storage protein genes on each of the group 1 and group 6 chromosomes are scattered or are clustered together. For example, Payne *et al.* (1983) crossed the variety Hope with Cappelle-

TABLE 2. CHROMOSOMAL LOCATION OF THE STORAGE PROTEIN GENES

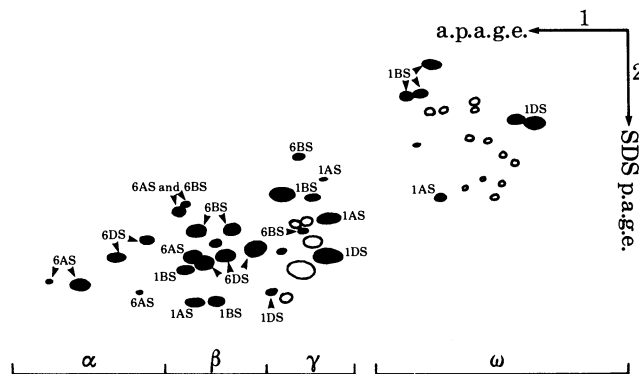| storage protein groups	chromosomes	arm
(1) h.m.m. glutenin subunits	1 A, 1 B, 1 D	long
(2) l.m.m. glutenin subunits ω-gliadins γ-gliadins β-gliadins (few)	1 A, 1 B, 1 D	short
(3) α-gliadins β-gliadins γ-gliadins (few)	6 A, 6 B, 6 D	short

FIGURE 2. Two dimensional fractionation of the gliadins of Chinese Spring (solid circles) and those of Cappelle-Desprez 1 A, Cheyenne 1 A, Hope 1 A, Timstein 1 A into Chinese Spring, Bezostaya 1 A into Cappelle-Desprez and Champlein 1 A into Bersée (open circles).

Desprez and the F_1 hybrid was crossed as female to Highbury; 348 progeny were analysed by SDS p.a.g.e. Six proteins, all coded by chromosome 1 A and inherited from the two primary parents, could be detected clearly in all progeny and were observed to be segregating. Three of the proteins were inherited from Hope, two of them were ω-gliadins and the other an γ-gliadin, the three from Cappelle-Desprez were an ω-gliadin and two l.m.m. subunits. All progeny had either the block of three proteins from Hope or Cappelle-Desprez: in none of the offspring did a recombination occur, giving rise to a mixture of these chromosome 1 A proteins from both parents. From this and other crosses it was concluded (Payne *et al.* 1983) that the storage protein genes that occur on the same chromosome arm are always clustered together at the same locus within about 1 cM of each other. These conclusions are strongly supported by the earlier and more extensive analyses of α-, β-, γ- and ω-gliadins (though not glutenin subunits) by Sozinov and colleagues in the U.S.S.R. (Sozinov & Poperelya 1980).

To summarize, storage protein genes occur at just nine different, complex loci in the wheat genome. The loci *Glu-A1*, *Glu-B1* and *Glu-D1* code for h.m.m. subunits of glutenin and they

are carried on the long arms of chromosomes 1 A, 1 B and 1 D. The loci *Gli-A1*, *Gli-B1* and *Gli-D1*, which occur on the short arms of chromosomes 1 A, 1 B and 1 D, are far more complex because each contains three families of genes coding for three protein groups: ω-gliadins, γ-gliadins and l.m.m. subunits of glutenin. These proteins have distinctive biochemical properties (Jackson *et al.* 1983) and different N-terminal sequences (Shewry *et al.*, this symposium) so their genes at best can only be distantly related. The three remaining loci on the short arms of the group 6 chromosomes, *Gli-A2*, *Gli-B2*, and *Gli-D2* have an intermediate complexity between *Glu-1* and *Gli-1*. Their genes code for α- and β-gliadins that all appear to have related N-terminal amino acid sequences (Bietz *et al.* 1976).

It is presumed that the variation seen in different varieties is due to the presence of allelic genes at each of the nine storage protein loci. However, since recombination between genes at any individual locus is rare, this is very difficult to prove.

Gene mapping

Lawrence & Shepherd (1981 *b*) were the first to estimate recombination between *Glu-1* and *Gli-1* genes on the same chromosome. They analysed the progeny of a tri-parental cross, the parents having different, allelic h.m.m. glutenin subunits and ω-gliadins controlled by chromosome 1 B, and estimated recombination between *Glu-B1* and *Gli-B1* to be 48.8%. As this value was not significantly different from 50%, the authors concluded that the two gene loci segregated independently. Later, Payne *et al.* (1982 *b*) obtained recombination values between *Glu-A1* and *Gli-A1* of 47% and 42% and between *Glu-B1* and *Gli-B1* of 39% and 47% in four different crosses. When the four percentages were compared by a heterogeneity χ^2, they were shown not to be significantly different from each other. The data was therefore pooled to obtain a combined estimate of recombination between *Glu-1* and *Gli-1* on chromosomes 1 A and 1 B. From 1208 classifications, linkage at 43% was significant. However, in a further study with F_2-selfed progeny, an independent segregation was found between *Glu-D1* and *Gli-D1*, recombination being 48.3% (Chojecki *et al.* 1983). The conclusions to be drawn from these experiments are that *Glu-1* on the long arm of the group 1 chromosomes are distantly separated from *Gli-1* on their respective short arms, with either very little or no significant linkage between them.

By analysing 399 progeny of crosses made with ditelocentric lines, Rybalka & Sozinov (1979) demonstrated a 42% recombination between the centromere of chromosome 1 B and locus *Gli-B1*: this showed that the latter must be located distally on the short arms. If it is assumed that a weak linkage does occur between *Glu-1* and *Gli-1*, then it follows that *Glu-1* must be located on the long arm close to the centromere. Direct evidence for this was obtained by Payne *et al.* (1982 *b*) by telocentric mapping. They obtained recombination percentages of 7.6, 9.2 and 10.1 between *Glu-A1*, *Glu-B1* and *Glu-D1* and the relevant centromeres, respectively.

Recently, P. I. Payne and L. M. Holt (unpublished findings) mapped the *Gli-A2* locus on the short arm of chromosome 6 A relative to the centromere. A recombination percentage of 35.5 was obtained by telocentric analysis showing that, as with *Gli-1* on the group 1 chromosomes, the locus is carried distally. The relative positions of all the storage-protein loci are illustrated in figure 3.

During a recombination study between *Glu-B1* and *Gli-B1*, a spontaneous mutant was detected that completely lacked all the *Gli-B1* storage proteins. Light microscopy of metaphase

nuclei of root-tip cells after Feulgen staining revealed that the satellite segment of chromosome 1BS was also lacking although at least a part of the short arm was still present. Two further types of study were undertaken with this mutant: light microscopy of chromosomes after *in situ* hybridization with radioactive ribosomal RNA genes cloned in plasmids (J. Hutchinson & R. B. Flavell, unpublished findings) and low-power electron microscopy of serial sections of
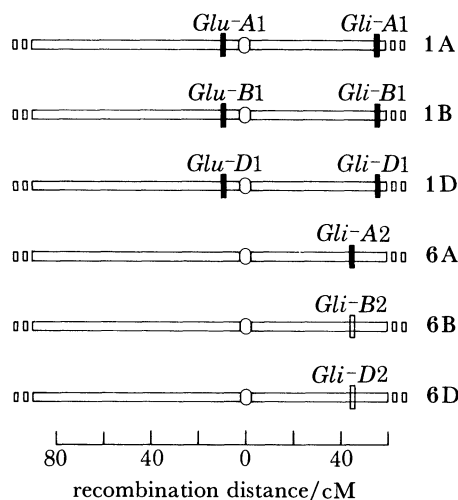

FIGURE 3. Chromosomal location of the storage protein genes in wheat. The location of the open rectangles designating loci for *Gli-B2* and *Gli-D2* was not determined by direct experimentation but by analogy from the results with *Gli-A2*. The distance between the centromere and *Gli-1* is not significantly different from the distance between the centromere and *Gli-A2*.

metaphase, mitotic nuclei (M. D. Bennett, unpublished). Both approaches indicated that chromosome 1B of the mutant had broken within the nucleolar organizing region, with the satellite completely lost and the rest of the short arm remaining. Thus, locus *Gli-B1* must occur in the satellite of chromosomes 1B that amounts to about one third of the total arm (Riley *et al.* 1958). Since the satellite of chromosome 6B consists of one third to one half of the short arm (Riley *et al.* 1958) it is likely from the telocentric recombination data above that *Gli-B2* is located similarly in this chromosome segment. Currently, the frequency of recombination between ribosomal RNA genes and *Gli-B1* on chromosome 1B and between equivalent genes on chromosome 6B is being determined (P. I. Payne, J. W. Snape, L. M. Holt & R. B. Flavell, unpublished). Preliminary data show moderate recombination for both chromosomes, which indicates that gliadin loci must be very close to the ends of the chromosome arms.

Work is in progress at the Plant Breeding Institute to map in more detail the storage protein genes by the assessment of recombination with other genes. For instance *Glu-B1* and *Gli-B1* are being linked to lectin genes on the long arm of chromosome 1B and to genes determining the following characters on the short arm: red glume (*Rg-1*), ribosomal RNA, male-sterility restorers and yellow rust resistance (*Yr10*).

STORAGE PROTEIN VARIATION AND BREAD-MAKING QUALITY

In the U.K. a major objective of wheat breeders is to develop new, high yielding varieties with improved bread-making qualities. Quality wheats have traditionally been imported from Canada and home-grown wheat used for biscuit and cake manufacture and in animal feed.

Through changed economic circumstances, particularly as a result of the U.K. joining the E.E.C., home-grown bread-quality wheats are in great demand for the first time.

Wheat for making bread must have certain minimum levels of protein content and protein quality. Above these minima, an increase in content, or quality, or both, dramatically improves the volume and texture of baked loaves (Finney & Barmore 1948).
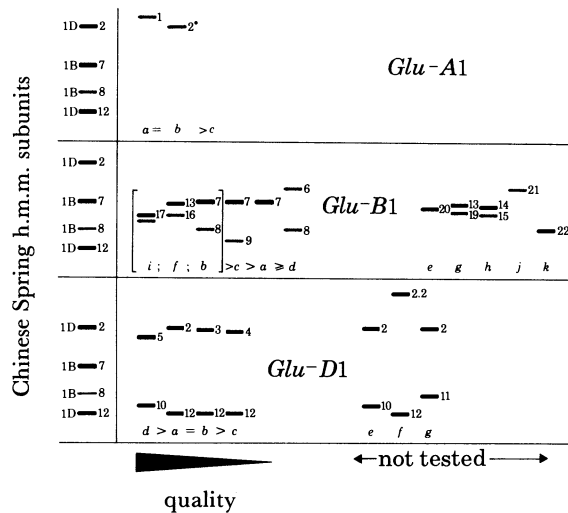

FIGURE 4. Allelic variation in the h.m.m. subunits of glutenin and their relationship to bread-making quality. On the left hand side are the standard h.m.m. subunits from Chinese Spring. The subunits have been split into three groups according to whether their genes are controlled by chromosome 1 A, 1 B or 1 D. The letter beneath each allelic group refers to the allele designation previously published (Payne & Lawrence 1983). The subunits that associate most strongly with good quality have been placed on the left hand side of each column.

Unfortunately, in cereal crops, there is generally an inverse relationship between grain yield and grain protein content. In addition, grain protein content is influenced more by the environment and fertilizer applications than by genotype. Thus, it is a difficult, though not an impossible task, for the wheat breeder to increase grain protein content without incurring a yield penalty. At the Plant Breeding Institute, a greater effort is being placed on the improvement of protein quality in the breeding programmes. Differences between the protein quality of varieties is considered to be caused by different combinations of storage protein variants that are present in the grain. The storage protein composition of a variety is independent of grain protein content (J. R. S. Ellis, this symposium) and therefore protein quality is largely independent of the environment. However, it is likely to be inherited in a complex manner if most, or all the storage protein loci are involved.

To determine which storage protein loci show significant variation for protein quality, and which alleles in particular are associated with good quality, the progeny of many crosses between parents with contrasting bread-making qualities and storage-protein variants were analysed. For each cross, between 100 and 150 F_2-selfed progeny were each bulked at the F_3 generation and tested for protein type by SDS p.a.g.e. and for protein quality by the SDS sedimentation test of Axford et al. (1979). As described previously (Payne et al. 1979, 1981) significant associations were found for certain h.m.m. subunits of glutenin and a summary of our current knowledge is shown in figure 4. In further work, segregating progeny have also

been analysed by a.p.a.g.e. to study gliadin variants in relation to protein quality. This work is still in progress but the preliminary results suggest that certain allelic variants coded by the group 1 loci are associated with good protein quality, though the degree of association was less than that detected for some of the h.m.m. glutenin subunits. The two major variant blocks of gliadins coded by chromosome 6A did not significantly correlate with either good or poor quality. Unfortunately the chromosome 6B and 6D variants could not be tested because of inadequate resolution by a.p.a.g.e. Consequently the progeny of one cross, between the varieties Alcedo and Brigand, are being analysed by two dimensional a.p.a.g.e $\times$ SDS p.a.g.e. as well as SDS p.a.g.e to reveal allelic variation and quality associations at eight of the nine storage protein loci. Both parents carry the null allele for *Glu-A1* so this locus could not be assessed. The results so far support our other findings on the relative importance of different alleles at the storage protein loci: i.e. *Glu-1 > Gli-1 > Gli-2*.

Our results on the relationship between allelic variation in h.m.m. subunits of glutenin and bread-making quality are generally supported by those of Burnouf & Bouriquet (1980) and Moonen *et al.* (1982). Quality associations were also found among allelic gliadin and glutenin proteins by Wrigley *et al.* (1982), although they concluded that associations were strongest for the gliadins, particularly bands 2, 4, 14 and 19. As all these will be coded by *Gli-1* rather than *Gli-2*, the following relationship may hold: *Gli-1 > Glu-1 > Gli-2*. Sozinov & Poperelya (1980) analysed segregated progeny of many crosses for associations between bread-making quality and gliadin type and found associations for all the *Gli-1* and *Gli-2* loci. However, in agreement with our work, the relative importance of these two groups of loci is *Gli-1 > Gli-2* (A. A. Sozinov, personal communication).

In a previous section, it was shown that the *Gli-1* locus is complex, coding for ω-gliadins, γ-gliadins and l.m.m. subunits of glutenin. Because these genes do not recombine during the formation of segregating progeny, it cannot be determined directly which group or groups are causing the difference in protein quality for bread-making. However, as the SDS-sedimentation test measures protein quality by determining the extent of protein gel formation in solutions of SDS and lactic acid, which is a function of glutenin, not gliadin (Moonen *et al.* 1982), then this would implicate the l.m.m. subunits.

Thus, differences in protein quality for bread-making that occur between varieties is envisaged as being mainly caused by different combinations of both h.m.m. and l.m.m. glutenin subunits. As genes for these do not occur on the group 6 chromosomes, then it would be predicted that different alleles at loci on these chromosomes confer only minor differences in bread-making quality, and, from our work, this would appear to be the case.

To analyse in more detail the involvement of different proteins in protein quality for bread-making, near-isogenic lines of the variety Sicco are being developed. In one such line, Chinese Spring was crossed to Sicco to incorporate the genes for subunits 2 and 12 (*Glu-D1*). The F_1 offspring were backcrossed to Sicco and the embryoless halves of grains at the next generation were analysed by SDS p.a.g.e. The embryos of those containing subunits 2 and 12 were grown and backcrossed again to Sicco. When at the fifth backcross, selected grains should be genetically very similar to Sicco and these will be selfed. Grains homozygous for subunits 2 and 12 (and thus lacking the allelic subunits, 5 and 10, found in Sicco) will then be bulked and the flour used for tests of bread-making quality. From figure 4 it would be expected that this isogenic line would have inferior quality to Sicco. Several near isogenic lines, each

containing different kinds of introduced proteins, are currently being developed. Eventually pairs of isogenics will be crossed together and offspring homozygous for both of the introduced proteins will give, for the first time, information on the effect of protein–protein interactions on quality.

Although the benefits to the wheat breeder will be mainly for the future there is some scope for capitalizing on current information. Thus Plant Breeding Institute quality wheats all have the good quality allele at *Glu-A1* coding for subunit 1 (Payne *et al.* 1979) but they are deficient in other good quality h.m.m. glutenin subunits (Payne *et al.* 1981). The most recent varieties, Bounty and Avalon, are being crossed with suitable, western European wheats that contain subunits 5 and 10, the prime chromosome 1D subunits (figure 4). Progeny that are doubly homozygous for both subunits 1 and 5/10 should have better quality than either parent.
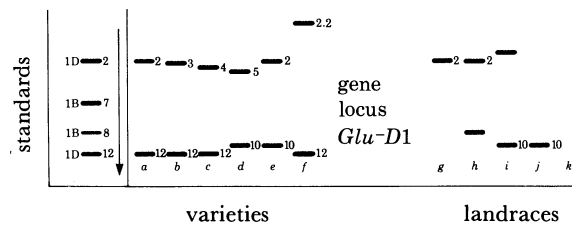

FIGURE 5. Variation in the h.m.m. subunits of glutenin coded by chromosome 1D determined in the analysis of modern varieties and ancient landraces from: allele *g*, Nepal; allele *h*, Yugoslavia and Iran; allele *i*, Iran; allele *j*, Afghanistan and Iran; allele *k*, India.

NEW SOURCES OF STORAGE PROTEIN VARIATION

Outside the genetically uniform wheat varieties of the developed countries, there is a vast gene pool in the landraces of primitive agriculture. Landraces are particularly prevalent and varied in countries that border the Mediterranean Sea and in the foothills of the Himalayan mountains. Generally, a landrace from a particular area will consist of a mixture of genotypes, and will show variation in many characters, including plant form, and will have been fashioned by natural selection, and to a limited extent by the farmer. With respect to endosperm storage proteins, mutations of their genes would be expected to be both tolerated and perpetuated, provided there were no associated adverse effects. To survey this variation, collections of landraces are being screened for protein type. Many new forms have been detected, even in the small sample of landraces analysed so far. The genes for some of them will be transferred to the Sicco genome to test their effect on technological properties such as bread-making quality, and on agricultural performance, such as yield.

As an example of this variation, new h.m.m. subunits of glutenin thought to be controlled by chromosome 1D are shown in figure 5 and compared to those previously found in modern varieties. As well as variation in the size of subunits, there is also variation in the number of subunits expressed. The normal number is two, one each of the 1Dx and 1Dy groups (Payne *et al.* 1981). Lines were detected where one of the subunits, 1Dx in some cases and 1Dy in others, was apparently deleted, perhaps by a mutation in the initiator sequence of the structural gene. Single-gene deletions of this kind may well confer significant changes on the properties of gluten but this is unlikely to be the case for the *Gli-1* and *Gli-2* loci for they each contain many more structural genes and the loss of one would be insignificant. However as these loci occur near the ends of chromosomes (figure 3) it is possible that a chromosome break could delete the whole

locus. This is known to have occurred in the spontaneous 1 BS satellite deletion discussed in a previous section. It is not found in landraces because the depleted chromosome is associated, unfortunately, with poor seed set. However a French variety, Darius, and the majority of landraces from eastern Nepal do not produce any proteins coded at the *Gli-D1* locus – ω-gliadins, γ-gliadins and l.m.m. glutenin subunits – and it has been speculated that this is because of a deletion of a very small piece of chromosome 1D (P. I. Payne, L. M. Holt, E. A. Jackson, A. J. S. Chojecki, M. D. Gale and M. D. Bennett, unpublished results). As in this case fertility is not reduced, this *Gli-D1* null is being transferred to Sicco for assessment. New variant forms have been found for gliadins coded by the group 6 chromosomes but as yet, no nulls.

An alternative source of genes coding for novel proteins are the diploid and tetraploid wild relatives of wheat. Variation here is greater still than in the landraces (Law & Payne 1983) because of their great antiquity and it also follows that there will be greater differences in protein structure. The major difficulty of this approach is that the chromosomes of many diploids, particularly the distant relatives, will not normally recombine with those of bread-wheat. Fortunately, this can be obviated by crossing the wheat relatives to Chinese Spring N5B T5D as chromosome 5B contains the *Ph* gene that prevents recombination between homoeologous chromosomes (Riley & Chapman 1958). By using this approach the *Gli-1* locus of *Aegilops umbellulata* has been successfully transferred to bread wheat. In two lines it was incorporated into chromosome 1B with the elimination of *Gli-B1* and in a third line it was incorporated into chromosome 1D (P. A. Harris, P. I. Payne and C. N. Law, unpublished).

Conclusions

Genetic analysis of the endosperm storage proteins in bread wheat is beginning to be exploited by the plant breeder to develop new varieties with improved bread-making quality. The range of allelic variation in storage proteins that is currently available to the plant breeder is being extended by the introgression of alleles from primitive landraces and from alien wheats. Linkage studies between storage protein genes and genes for other characters might enable the incorporation of desired traits into new wheat varieties, such as improved cold tolerance and disease resistance (Sozinov 1981), by electrophoretic screening.

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Discussion

A. B. DAMANIA (*Department of Plant Biology, Birmingham University, U.K.*). I have worked extensively on morphological and electrophoretic variations in wheat landraces from Nepal. It was found that about six landraces out of a total of 16 studied have several seeds where the ω-gliadin bands were absent. The landrace from Lomson, of which Dr Payne showed a slide, was an extreme example where 48 profiles out of the 50 single seeds I selected at random, lacked the bands coded by genes located on the short arm of the 1 D chromosome of the hexaploid wheat. In other landraces a varying number of extracts of single seeds had missing bands. I would like to ask Dr Payne if there is any mechanism he knows that influences the frequencies of occurrence of these chromosomal deletions or suppression of the expression of genes among seeds of wheat landraces from Nepal.

P. I. PAYNE. We have analysed four of A. B. Damania's landrace samples from Nepal and agree with his conclusions. We have also analysed 60 other landraces from East Nepal that were collected at different locations by the University College Bangor Nepal Expedition, 1971 (Whitcombe, J. R. (1975), Cereals in Nepal, *Pl. Genet. Resources Newsl. F.A.O.* **31**, 19–28). The incidence of deleted chromosome 1 D gliadins and low molecular mass glutenin subunits is much higher and occurred in about 90 % of the grains tested. However, none of the landraces analysed from neighbouring countries to the west (northern Pakistan and India) and to the north (Tibet) have such deletions and this presumably reflects the past isolation of Nepal from its neighbours.

It is likely that the mutation that gave rise to deleted chromosome 1 D gliadins was a very rare ancient occurrence and probably happened once or on a very limited number of occasions.

I do not feel that there is necessarily any great significance in different landraces having different proportions of chromosome 1 D gliadin deletions. The phenomenon can be regarded as being equivalent to an allelic variant and I am sure that different allelic variants of storage proteins coded by other chromosomes will also occur at different frequencies in different landraces. However, if it is assumed that the missing storage proteins are caused by the loss of a small, terminal piece of chromosome 1 D that includes *Gli-D1*, then other, linked genes will also be lost. Some of them could affect agronomic performance or plant form and so influence the farmers selection for the next growing season. The *Gli-D1* null form of Lomsom is currently being transferred to Sicco by recurrent backcrossing and selection. The effects on Sicco of any deleted, linked genes should then be revealed.
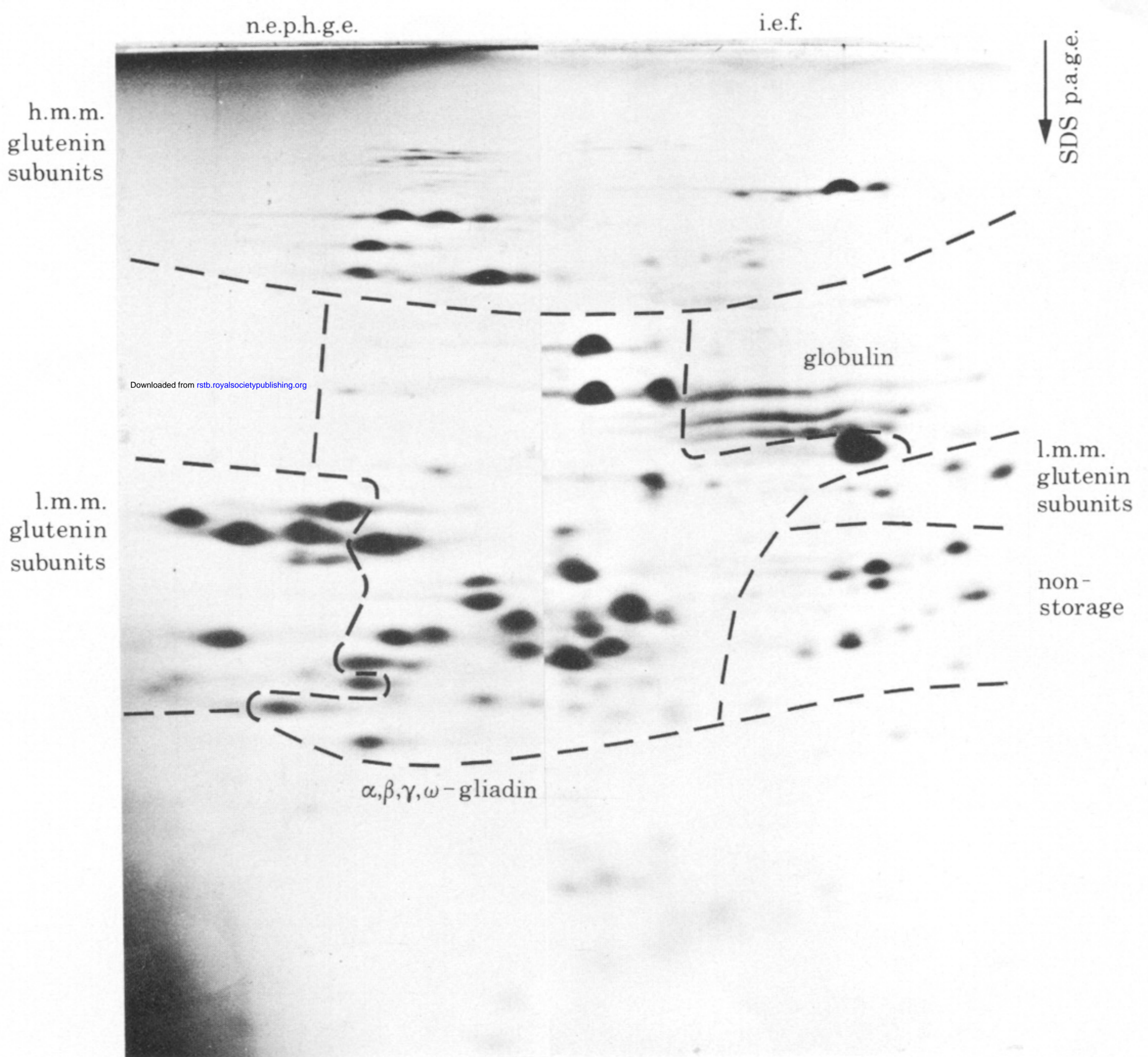
FIGURE 1. Fractionation of the total endosperm proteins of the variety Chinese Spring by two different two dimensional electrophoresis systems: n.e.p.h.g.e. × SDS p.a.g.e. and i.e.f. × SDS p.a.g.e. The distinction between glutenin subunits and gliadins was determined by gel filtration chromatography and the identification of globulin proteins was by their solubility in 0.5 M sodium chloride.