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Grain Protein Variability Among Species of *Triticum* and *Aegilops*: Quantitative SDS-PAGE Studies

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Summary. Total proteins were extracted from degermed seeds of various species of *Triticum* and *Aegilops* with solutions containing sodium dodecyl sulfate (SDS) and mercaptoethanol. The reduced, dissociated proteins were fractionated according to molecular weight (MW) by high-resolution polyacrylamide gel electrophoresis in buffers containing SDS (SDS-PAGE). Stained SDS-PAGE patterns were measured by densitometric scanning over a suitable range of optical density. The data were normalized to equivalent total areas for each of the densitometric scans by means of a computer program that also permitted the construction of patterns of hypothetical amphiploids by averaging patterns of two or three diploid species. The grain proteins of most species examined had distinctive qualitative and quantitative aspects that were characteristic of the species even though nearly every accession or cultivar of a species exhibited at least minor differences in pattern from other accessions or cultivars. The main protein components (probably prolamins) of *Triticum monococcum* ssp. *monococcum*, *T. monococcum* ssp. *boeoticum*, *T. urartu*, and *Aegilops squarrosa* had MW's in the range 29-36 $\times 10^3$ whereas the most important components of *Ae. speltoides*, *Ae. longissima*, and *Ae. searsii* had MW's in the range 37-55 $\times 10^3$. Changes in the quantitative expression of particular genes, especially those coding for storage protein components, may have been associated with speciation. The strong predominance of proteins with MW's in the range 29-36 $\times 10^3$ in some accessions of AB genome tetraploids, such as *T. turgidum* ssp. *dicoccoides*, may indicate contributions to the B genome of these tetraploids by *T. monococcum* ssp. *boeoticum*, *T. urartu*, or *Ae. squarrosa*.

Key words: Wheat proteins – Electrophoresis – *Triticum* – *Aegilops*

Introduction

Wheats of commercial importance are polyploid species that presumably incorporate the genomes of two (durum wheats – *T. turgidum* ssp. *turgidum* conv. *durum*, 2n = 4x = 28) or three (bread wheats – *T. aestivum* ssp. *vulgare*, 2n = 6x = 42) different diploid species of *Triticum* and *Aegilops*. Evidence has accumulated, however, that polyploid wheats have a more complex genetic makeup than would be expected from their ploidy levels as a consequence of the mechanism by which they were formed or because of introgression from other species subsequent to amphiploid formation (Harlan and deWet 1975; Zohary and Feldman 1962; Vardi 1973; Konzak 1977).

Seed proteins are products representative of many genes in the genomes of species of *Triticum* and *Aegilops* and their analysis can provide useful information about evolutionary relationships and genome complexity in these species (Konarev et al. 1976; Vitozzi and Silano 1976; Caldwell and Kasarda 1978). Analysis of seed proteins by extraction with sodium dodecyl sulfate (SDS) and mercaptoethanol followed by polyacrylamide gel electrophoresis (SDS-PAGE) is a method that has not yet been adequately explored in this regard. The migration of proteins in SDS-PAGE is mainly a function of the molecular weights (MW's) of their reduced, dissociated polypeptide chains. Although differences in amino acid compositions may bring about resolution of components that do not differ in MW (de Jongh et al. 1978; Noel et al. 1979), the distance of migration in the gel is still determining primarily by MW and any increased resolution resulting from compositional differences can be helpful in distinguishing complex patterns from one another.

In earlier related work, Preston et al. (1975), Bietz et al. (1975), and Dhaliwal (1977) compared SDS-PAGE patterns of various grain protein fractions or of total grain proteins from various species of *Triticum* and *Aegilops*. These investigators used methods for SDS-PAGE that were similar to that of Weber and Osborne (1969) and their analysis of patterns was mainly qualitative. Payne and Corfield (1979) and Payne et al. (1979) recently have demonstrated, however, that Laemmli's (1970) approach to SDS-PAGE provides much better resolution of wheat grain proteins than the Weber and Osborne (1969) method.

We have combined high-resolution SDS-PAGE (Payne and Corfield 1979) with our method for complete extraction of grain proteins with solutions containing SDS and mercaptoethanol (Fullington et al. 1980) to provide new information about relationships among diploid and polyploid species of *Triticum* and *Aegilops* through analysis

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of their grain proteins. In addition to using a method that gives higher resolution, we have (1) used slab gels that enabled us to include many samples on the same gel for better comparison of protein mobilities; (2) included MW standards on gels and included their patterns in most of our figures so that independent calibration and analysis of our results can be made; (3) examined multiple accessions or cultivars of most species in our study; (4) quantitated our SDS-PAGE patterns by scanning densitometry; (5) normalized patterns to equivalent area for comparison by means of a computer program that also permitted the construction of hypothetical polyploids from the patterns of diploid species; and (6) included additional species (*Ae. searsii*, *Ae. longissima*, and *T. zhukovskyi*) that had not been examined by other researchers.

Our results show that grain proteins of the different species we examined by SDS-PAGE have quantitative and qualitative characteristics that are usually distinctive for each species – although nearly every accession or cultivar had at least minor differences in patterns even within a species. These characteristics suggest that changes in quantitative expression of some genes, particularly those coding for storage protein components occurred in speciation of *Triticum* and *Aegilops*. We also interpret these characteristics as indicating that tetraploids of the AB genome type have more than two diploid progenitors that contributed significantly to their genetic makeup and that contributions to the B genome of AB-type tetraploids may have been made by A-genome types (such as *T. monococcum* ssp. *boeoticum* or *T. urartu*) or by *Ae. squarrosa*.

Materials and Methods

Plant Material

Each accession or cultivar (cv.) examined is listed according to species and numbered successively; each number is followed by any identifying code provided by the supplier of the seed and by the geographical location of its origin when that information was available. Nomenclature is based on the proposal of Mac Key (1963). Hexaploid species (6x), cultivated. *T. aestivum* ssp. *vulgare*: no. 1 cv. 'Scout 66'; no. 2 cv. 'Justin'; no. 3 cv. 'Chinese Spring'; no. 4 cv. 'Atlas 66'. *T. aestivum* ssp. *spelta*: no. 1 G524. *T. zhukovskyi*: no. 1 G986. Tetraploid species (4x), cultivated. *T. turgidum* ssp. *dicoccum*: no. 1 cv. 'Khapli emmer', 63-2356. *T. turgidum* ssp. *carthlicum* no. 1 PI 283888; no. 2 PI 283890. *T. turgidum* ssp. *turgidum* conv. *polonicum*: no. 1 PI 289606. *T. turgidum* ssp. *turgidum* conv. *durum*: no. 1 cv. 'Leeds'; no. 2 cv. 'Agathe'; no. 3 cv. 'Montferrier'.

Tetraploid species (4x), wild. *T. turgidum* ssp. *dicoccoides*: no. 1 G2107 (Turkey); no. 2 77-2-23-11 (Israel); no. 3 F28-8-3 (Israel); no. 4 G3074 (Lebanon); no. 5 64-1148. *T. timopheevi* ssp. *armeniacum* (*T. araraticum*): no. 1 G1761 (Transcaucasia).

Diploid species (2x), cultivated. *T. monococcum* ssp. *monococcum*: no. 1 UM. Diploid species (2x), wild. *T. monococcum* ssp. *boeoticum*: no. 1 G1758 (Transcaucasia); no. 2 G2512 (Iran); no. 3 G1804 (Turkey); no. 4 G3116 (Lebanon); no. 5 G642 (Turkey); no. 6 G2528 (Iran); no. 7 G1756 (Transcaucasia); no. 8 G1769 (Turkey); no. 9 G1773 (Turkey); no. 10 G2213 (Turkey). *T. urartu*: no. 1 G3151 (Lebanon); no. 2 G1876 (Turkey). *Ae. speltoides*: no. 1 G617; no. 2 UCD; no. 3 G1176. *Ae. longissima*: no. 1 G1304 (Israel); no. 2 F214-3-36 (Israel); no. 3 F213-1 (Israel); no. 4 FO11-F-UH (Israel); no. 5 G1306. *Ae. searsii*: no. 1 F-113-11 (Israel); no. 2 F101 (Israel); no. 3 F102 (Israel); no. 4 F103 (Israel). *Ae. squarrosa*: no. 1 64-1153; no. 2 0623; no. 3 G405; no. 4 64-1154; no. 5 138-8.

Solubility Fractions

Fractions separated by means of solubility differences were prepared from endosperm (flour) of the hard red winter wheat cv. 'Scout 66', which is commonly grown in the Midwestern United States. Albumins and globulins were extracted from the flour with 0.1 M sodium chloride. The supernatant was clarified by centrifugation and dialyzed thoroughly against distilled water. The small amount of precipitate that resulted was taken as the globulin fraction. The freeze-dried supernatant was taken as the albumin fraction. Gliadins were prepared by extracting flour with 70% (v/v) ethanol-water solution, clarifying the extract by centrifugation, and precipitating the gliadins from the supernatant by adding three times its volume of 1.5% sodium chloride. The precipitated gliadins were then dialyzed against distilled water, centrifuged, and the clear supernatant freeze-dried as the gliadin preparation. The residue remaining after gliadin extraction with ethanol-water was taken as the glutenin preparation. This residue includes starch and other carbohydrates as well as proteins and the proteins in the residue may differ somewhat from those in glutenin preparations based on solubilization of proteins from the residue: see Kasarda et al. (1976a). Protein contents were based on Kjeldahl nitrogen analyses (N X 5.7).

Gliadin Preparations

The gliadin preparations from *Ae. speltoides* no. 2 (the number refers to the code in Plant Material), *Ae. squarrosa* no. 3 and no. 5, *T. turgidum* ssp. *dicoccum* no. 1, *T. turgidum* ssp. *dicoccoides* no. 5, *T. monococcum* ssp. *monococcum* no. 1, and *T. urartu* no. 1 were prepared as described by Charbonnier (1970) and were the same ones examined by Autran et al. (1979). A-gliadin was prepared by the procedure of Bernardin et al. (1967).

Extraction of Proteins from Seeds

Total proteins for electrophoresis were extracted from de-germed seeds as described previously (Fullington et al. 1980) except that the extracting solvent was a mixture of 0.062 M Tris-HCl (pH 6.8) that included 2% SDS, 5% mercaptoethanol, and 0.01% Pyronin Y (tracking dye). After grinding the seeds with the solvent in a mortar and pestle, solid sucrose was added to the extent of 10%, and the mixture was centrifuged. Samples of the resulting supernatant ranging from 5 to 30 µl were taken for SDS-PAGE.

Electrophoresis

SDS-PAGE was carried out with a vertical gel apparatus (Hoefer Scientific Instrument Co., San Francisco) for 15 hrs at a current of 9 ma. Gel slabs were used throughout with 10 samples applied per gel. Separation gels (17 cm X 16.5 cm X 1.5 mm) contained

17.5% acrylamide and 0.08% N, N'-methylene-bis-acrylamide (bis) while the stacking gel (height 3.5 cm) contained 3% acrylamide and 0.043% bis. Molecular weight standards consisting of the following proteins were included on most gels: horse heart cytochrome C (12,500); ribonuclease (13,700); chymotrypsinogen A (25,000); ovalbumin (43,000); bovine serum albumin (68,000), and β-galactosidase (116,000) (Fowler and Zabin 1978). Some extraneous bands that appeared in the patterns of the standard mixture probably resulted from impurities in some of the preparations but presented no difficulties to calibration of the electrophoresis experiments. Each gel was calibrated by plotting log MW of the standards as a function of the distance moved in the gel. In those experiments where no standards were included, one of the samples had always been examined previously in an experiment that included standards so that it served as a secondary standard. Gels were stained for 24 hrs with 0.02% Coomassie Brilliant Blue R250 as described by Fullington et al. (1980). Gels were destained for 3 hrs in 6% trichloroacetic acid, restained, and then destained again for 16 hrs to enhance the intensity of the patterns. After the gels were destained, they were photographed through a red filter with Polaroid film Type 52 (black and white) or through a yellow-orange filter (Wratten G-15) with Polaroid film Type 108 (color); the latter method gave better results and the improvement was retained when the color prints were re-photographed with black and white film. After photography, each sample pattern was sliced from the gel, placed in a cuvette (20 cm long), and scanned densitometrically with a Gilford apparatus as described by Fullington et al. (1980) except that the scan rate was 1 cm per min and optical density was recorded at 10 cm per min. Previous work (Fullington et al. 1980) had indicated that peak areas changed linearly as a function of concentration when the difference in optical density between a peak and background was no more than 1 optical density unit; all of our patterns were within that range.

Quantitative Comparison of Patterns

A computer program written for a Tektronix 4051 computer was used to transform patterns for comparison. The program normalized the migration distance of each pattern to a reference peak (arbitrarily specified for the first set of data entered) and an equivalent point (determined by independent calibration of each gel) in each subsequent pattern. The program also made the total area defined by the densitometric scan of each pattern equivalent to that of the first pattern entered.

To aid discussion of quantitative aspects of the pattern, we have divided each pattern into five regions (areas), designated A1 through A5, corresponding to the following MW ranges: A1, 80 × 10^3 and higher; A2, 50-80 × 10^3; A3, 38-50 × 10^3; A4, 28-38 × 10^3; and A5, 10-28 × 10^3. These MW ranges differed slightly from those used in a previous study (Fullington et al. 1980) because the higher resolution of the technique used in this study resulted in a slightly different grouping of the major bands of the patterns. Henceforth, we shall substitute the abbreviation k for × 10^3 so that, for example, a MW of 80 × 10^3 will be written 80k.

Composite Patterns

The program used to normalize was also capable of producing composite patterns in which, after normalization, two or more patterns were summed and averaged. In this way a synthetic densitometric scan of a hypothetical amphiploid could be constructed from the scans of diploid species or lower-level polyploid species. Perfect register was difficult to obtain for all parts of the composite patterns in this way – different gels do not produce exactly the same function of MW in relation to migration distance even though differences were small. Consequently, the composites were useful mainly for interpreting gross quantitative characteristics.

Results and Discussion

Solubility Fractions from Scout 66

The SDS-PAGE patterns of albumins, globulins, gliadins, and glutenins (residue proteins) that had been separated by solubility differences from the common wheat cultivar 'Scout 66' are compared in Fig. 1 with the pattern of the total protein extract of the flour and with A-gliadin, a purified α-gliadin fraction (Bernardin et al. 1967; Platt et al. 1974) that also had been prepared from 'Scout 66'. The purpose of the comparisons was to aid in the interpretation of the SDS-PAGE patterns of total seed or endosperm proteins extracted from the various species of this study.

Samples sizes applied to the gel of Fig. 1 were chosen to give well-defined patterns and were not in proportion to the percentages of the total protein contributed by each of the solubility fractions. This must be kept in mind when comparing the patterns of the fractions with total protein patterns. The percentage of total protein that we found for each of the solubility fractions was as follows: albumins, 13%; globulins, 2%; gliadins, 29%; and glutenin (residue), 57%. Accordingly, the total protein patterns of Fig. 1b and g have received strong contributions from gliadins with MW's in the ranges of A4 (28k-

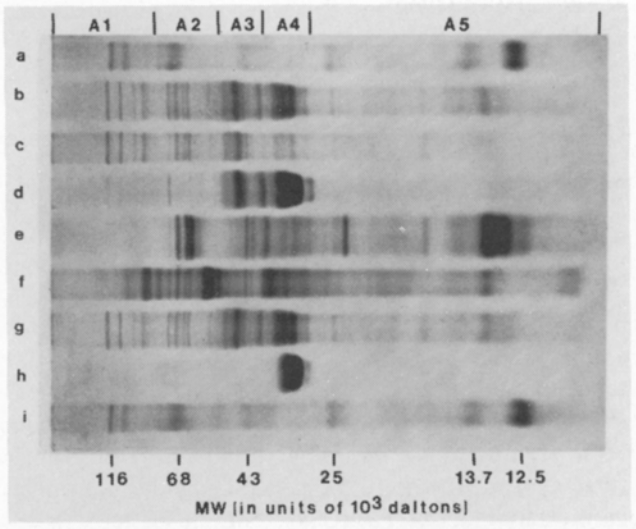

Fig. 1a-i. SDS-PAGE patterns of total proteins and solubility fractions of proteins extracted from *T. aestivum* ssp. *vulgare* cv. 'Scout 66': **a** standard proteins; **b** total proteins; **c** glutenins (residue proteins); **d** gliadins; **e** albumins; **f** globulins; **g** total proteins; **h** A-gliadin, **i** standard proteins for calibration of gel. Migration from left to right

38k) and A3 (38k-50k) and from glutenins with MW's in the range of A3 – with glutenins contributing importantly to A4, A2 (50k-80k), and A1 (80k and higher), as well. Albumins made their strongest contributions to the MW range of A5 (10k-28k) with especially strong bands near 13k. They may have contributed also to A2 insofar as the albumin fraction has three bands of medium intensity corresponding to MW's of 61.6k 63.1k, and 68.4k in its pattern; these bands appear to be equivalent to proteins with these MW's in the pattern of glutenins. Globulins probably contribute little to the total protein patterns inasmuch as they make up only 2% of the total protein. We have not examined all of the solubility fractions of diploid species, but on the basis of gliadins prepared from diploid species, we feel that the solubility fractions from 'Scout 66' may serve as a reasonable model for the proportions of solubility fractions in the diploids and for the likely associations of these fractions with the major bands of the SDS-PAGE patterns of total proteins extracted from diploid species.

Our high-resolution technique resulted in SDS-PAGE patterns rather different from those of most earlier investigations of wheat proteins by SDS-PAGE in which relatively low-resolution techniques were used (Bietz and Wall 1972; Jeanjean and Feillet 1978; Fullington et al. 1980), but either technique leads to similar conclusions regarding the distribution of protein components in the patterns of solubility fractions. In contrast to earlier work (Platt et al. 1974) in which A-gliadin gave only single band, the present method exhibited higher resolution, so that A-gliadin appeared as two major bands corresponding to MW's of 29k and 31k and a minor band corresponding to 27k. These MW's are somewhat lower than that estimated previously (Platt et al. 1974).

Gliadins from Selected Diploid and Tetraploid Species

As evident in the patterns of Fig. 1, gliadins contribute most importantly to the SDS-PAGE patterns of total protein extracts. To illustrate the differences among gliadin patterns of different species, we compared gliadins from several diploid species, including two different accessions of *Ae. squarrosa* with gliadins from the tetraploids *T. turgidum* ssp. *dicoccum* and ssp. *dicoccoides* and the hexaploid *T. aestivum* ssp. *vulgare* ('Scout 66'); patterns are shown in Fig. 2. Most of these gliadins patterns (Fig. 2) showed the greatest amounts of proteins in the range of A4 (28k-38k), but the patterns varied moderately. *Ae. speltoides* (Fig. 2a) differed from the other species compared in the figure in having most of its gliadin protein in the range of A3 – more specifically with MW's close to 43k. Insofar as we know, this is the first evidence that species of *Triticum* and *Aegilops* may

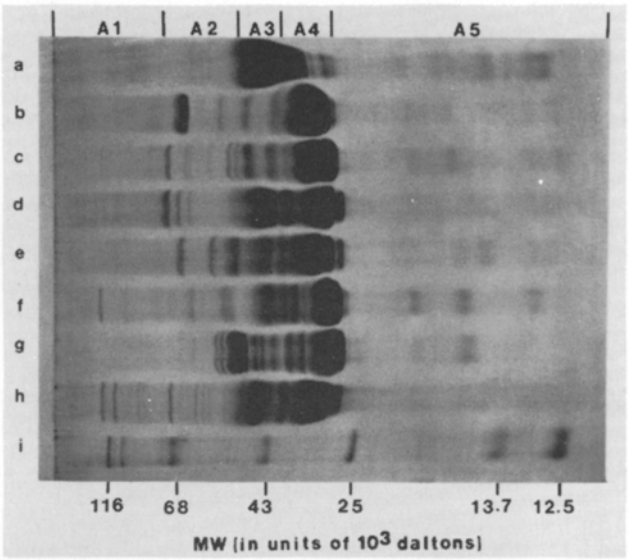

Fig. 2a-i. SDS-PAGE patterns of gliadins from different species and accessions: **a** *Ae. speltoides* no. 2; **b** *Ae. squarrosa* no. 4; **c** *Ae. squarrosa* no. 2; **d** *T. turgidum* ssp. *dicoccum* no. 1 (Khapli emmer); **e** *T. turgidum* ssp. *dicoccoides* no. 5; **f** *T. monococcum* ssp. *monococcum* no. 1; **g** *T. urartu* no. 1; **h** *T. aestivum* ssp. *vulgare* no. 1 ('Scout 66'); **i** standard proteins. Migration from left to right

differ importantly in the MW's of their major gliadin components. As reported by Autran et al. (1979), however, these species do not differ much in their N-terminal amino acid sequences; all those examined had both α-type and γ-type sequences accounting for 70-80% of their prolamins.

The patterns of Fig. 2 were obtained with concentrated samples. A more lightly loaded gel (not shown) was better for estimating MW's of the gliadin components. From this gel, we estimated that *squarrosa* no. 5 (Fig. 2b) had a major gliadin component with MW 34.3k whereas *squarrosa* no. 2 (Fig. 2c) had two intense bands in its gliadin pattern that corresponded to proteins with MW's of 30.6k and 35.1k. Both *T. urartu* no. 1 (Fig. 2g) and *T. monococcum* ssp. *monococcum* (Fig. 2f) had their most intense gliadin bands corresponding to a MW of 29.9k, whereas *T. urartu* no. 1 also had a moderately intense series of bands corresponding to MW's near 50k.

Ssp. *dicoccoides* and ssp. *dicoccum* are usually considered as wild and cultivated forms, respectively, of *T. turgidum*; it seems likely that common bread wheats (*T. aestivum* ssp. *vulgare*) resulted from crossing of ssp. *dicoccum* and *Ae. squarrosa* (followed by doubling of the chromosome number) (Jaaska 1978). The patterns of ssp. *dicoccoides* no. 5 (Fig. 2e) and ssp. *dicoccum* no. 1 (Fig. 2d) differed from one another slightly in A4 in that the major component of the former had a MW of 31.6k whereas that of the latter was 30.3k. This difference is definitely significant when obtained from compari-

sons made on a single gel, but might not be if the comparison had required MW estimations to be made on separate gels insofar as we found that MW calibrations based on standard proteins varied slightly from gel to gel.

Ssp. *dicoccum* no. 1 also differed from ssp. *dicoccoides* no. 5 importantly in having a component with MW 45.7k in A3 that was nearly as intense in its pattern as the component with MW 30.3k. The component with MW 45.7k may have been derived from a species such as *Ae. speltoides* in formation of the tetraploid. Both ssp. *dicoccum* no. 1 and ssp. *dicoccoides* no. 5 had gliadin components with MW's near 68k that are present in gliadins from *squarrosa* (Fig. 2b, c), but which are absent from or faint in the patterns of ssp. *monococcum*, *urartu*, and *speltoides* gliadins. These bands are probably derived from ω-gliadins. The pattern of gliadins from 'Scout 66' (*T. aestivum* ssp. *vulgare*) (Fig. 2h) was quite similar to that of gliadins from *T. turgidum* ssp. *dicoccum* no. 1 in agreement with the possibility that the AB genomes of ssp. *vulgare* (genome composition ABD) may have been contributed by the cultivated form (Jaaska 1978) of the tetraploid.

'Scout 66' gliadins showed the greatest complexity in A4 compared with the gliadins from the other species (although this was evident only in the more lightly loaded gels) as might be expected from the addition of components from all three genomes in this region. On a gel with light sample loading, we could distinguish six bands in the range of A4 – five were of moderate intensity and corresponded in MW to the range 30.9k to 36.7k while the sixth was less intense and corresponded to a MW of 28.8k. The important component in 'Scout 66' gliadins that corresponded in MW to 46.2k is probably the same as the component in ssp. *dicoccum* no. 1 (Fig. 2d) that we assigned a MW of 45.7k; this is within our error of measurement in this MW range for different samples on the same gel, particularly when they are not in adjacent tracks.

The highest-MW components found in the pattern of the total protein extract from 'Scout 66' (Fig. 1b) and in the pattern of glutenin from the same cultivar (Fig. 1c) are also present in the gliadins (Fig. 2h); the two slowest moving bands correspond to proteins with MW's of 120k and 105k. These high-MW components are evidently soluble to some degree in ethanol-water solutions and precipitate from salt solutions like gliadins; they are probably storage proteins.

Intra- and Interspecific Variations

Examples of SDS-PAGE patterns obtained from total protein extracts of several different diploid species are compared with patterns of total proteins from ssp. *dicoccum* no. 1 and 'Scout 66' in Fig. 3. All the patterns in Fig. 3 differed from one another and apparently provide a basis for analyzing relationships of diploid species to polyploid species. Before such an analysis could be carried out with any confidence, however, it was necessary to determine the extent to which variations occur in the total protein SDS-PAGE patterns of different accessions or varieties of a single species.

We examined the pattern diversity of *T. monococcum*

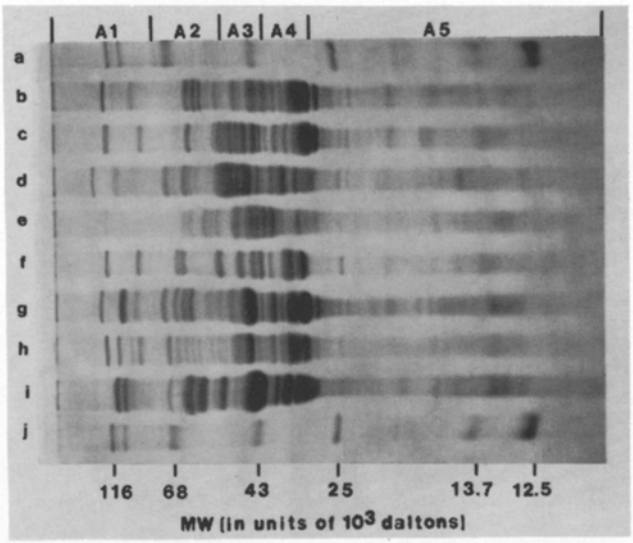

Fig. 3a-j. SDS-PAGE patterns of total proteins extracted from various species: **a** standard proteins; **b** *T. monococcum* ssp. *boeoticum* no. 2; **c** *T. urartu* no. 1; **d** *Ae. longissima* no. 1; **e** *Ae. speltoides* no. 2; **f** *Ae. squarrosa* no. 3; **g** *T. turgidum* ssp. *dicoccum* no. 1 ('Khapli emmer'); **h** *T. aestivum* ssp. *vulgare* no. 1 ('Scout 66'); **i** *Ae. searsii* no. 1; **j** standard proteins. Migration from left to right

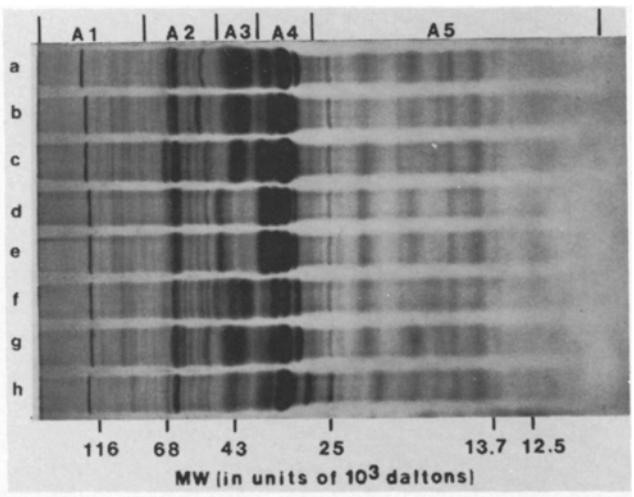

Fig. 4a-h. SDS-PAGE patterns of total proteins extracted from different accessions of *T. monococcum* ssp. *boeoticum*: **a** no. 5; **b** no. 7; **c** no. 1; **d** no. 8; **e** no. 9; **f** no. 3; **g** no. 10; **h** no. 4. Migration from left to right

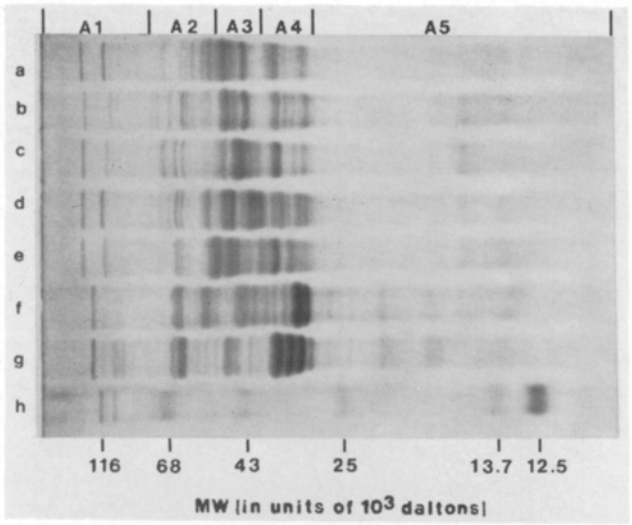

Fig. 5a-h. SDS-PAGE patterns of total proteins extracted from accessions of *Ae. longissima*, *T. monococcum* ssp. *monococcum* and *T. monococcum* ssp. *boeoticum*: **a** *Ae. longissima* no. 1; **b** *Ae. longissima* no. 4; **c** *Ae. longissima* no. 3; **d** *Ae. longissima* no. 2; **e** *Ae. longissima* no. 5; **f** *T. monococcum* ssp. *monococcum* no. 1; **g** *T. monococcum* ssp. *boeoticum* no. 8; **h** standard proteins. Migration from left to right

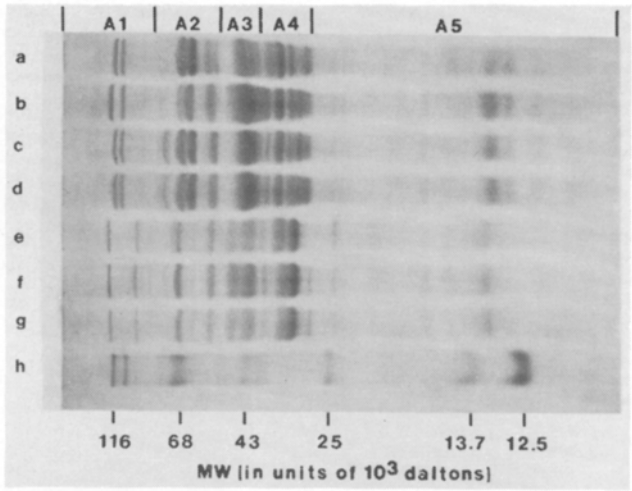

Fig. 6a-h. SDS-PAGE patterns of total proteins extracted from *Ae. searsii* and *Ae. squarrosa*: **a** *Ae. searsii* no. 2; **b** *Ae. searsii* no. 3; **c** *Ae. searsii* no. 4; **d** *Ae. searsii* no. 1; **e** *Ae. squarrosa* no. 3; **f** *Ae. squarrosa* no. 1 (seed from Univ. Nebraska); **g** *Ae. squarrosa* no. 1 (seed increased at WRRC). Migration from left to right

ssp. *boeoticum* (the wild form) most extensively – comparing ten different accessions; the patterns of eight accessions are shown in Fig. 4 and the pattern of ssp. *monococcum* (the domesticated form) is compared with ssp. *boeoticum* accession no. 8 in Fig. 5f and g. All the accessions of ssp. *boeoticum* (and ssp. *monococcum*) had similar patterns (compare the similarity of patterns in in Fig. 4 with the pattern variability evident in Fig. 3) although each differed from that of every other accession with the exception of numbers 8 and 9 (Fig. 4d, e). The pattern of proteins from these two latter accessions could not be distinguished from one another. Both were collected originally in Turkey and are identical on the basis of their SDS-PAGE patterns of total proteins.

The patterns of different accessions of ssp. *boeoticum* and ssp. *monococcum* were similar in their quantitative as well as qualitative aspects. The most intense bands of the patterns were contributed by components with MW's in the range of A4. This is illustrated by the example in Fig. 9c, which shows the tracings of the densitometric scan of proteins from ssp. *boeoticum* no. 2. These intense bands in ssp. *boeoticum* usually corresponded to proteins with MW's in the lower part of the range of A4 (28k-38k), frequently having MW's of 29k and 30k, but in some accessions (Fig. 4d) they extended as high as 36k.

We examined only two accessions of *T. urartu* (Figs. 3c, 8c), but their patterns were much the same as those of *T. monococcum* ssp. *boeoticum* and ssp. *monococcum* in quantitative aspects – for example see Fig. 8i. The patterns of the proteins of *urartu* differed qualitatively from those of ssp. *boeoticum* and ssp. *monococcum* in the MW range of A5, which corresponds mainly to albumins. *T. urartu* seems to be in the process of becoming reproductively isolated from *T. monococcum* (Johnson and Dhaliwal 1976), but the two species are closely related.

The patterns of total proteins from five different accessions of *Ae. longissima* are compared in Fig. 5 (a through e). As in the comparison of different accessions of *T. monococcum* ssp. *boeoticum* and ssp. *monococcum* strong common features are evident in these patterns despite differences that distinguish one from another. The most intense contribute to A3 (38k-50k) in contrast to those of ssp. *boeoticum*, ssp. *monococcum* and *urartu*, which were in A4 (28k-38k). Another distinguishing characteristic of *longissima*, not found in any other diploids were examined, was the presence of high-MW proteins in A1 with MW's of about 140k and 112k. The MW of the former component was significantly greater than the highest in other species. The bands corresponding to these two high-MW components in *longissima* were slightly displaced in one accession (Fig. 5b) so that they corresponded to MW's of 133k and 104k and the lower of the two was shifted slightly in several other accessions as well. These shifts may not represent true MW differences, but may result from compositional differences in the proteins, which have been noted to affect the mobilities of proteins in SDS-PAGE in a few cases (de Jongh et al. 1978; Noel et al. 1979).

The patterns of two accessions of *Ae. squarrosa* (numbers 1, 3) are compared in Fig. 6e, f and these patterns may be compared also with those of the gliadins from two other accessions (numbers 2, 4) in Fig. 2b and c. Again, patterns were distinctive for the species despite minor dif-

ferences among the accessions. In their quantitative aspects, *squarrosa* proteins (Fig. 9f) were similar to those of ssp. *boeoticum*, ssp. *monococcum* and *urartu* (Fig. 9c, b) with their most intense peaks in A4 although the most intense bands of *squarrosa* tended to be displaced toward the higher-MW end of the range with MW's of 32k-38k. None of our accessions of *squarrosa* showed the intense band that corresponded to a MW of 30k characteristic of most accessions of ssp. *boeoticum*, ssp. *monococcum* and *urartu*. We also compared the pattern of proteins from *squarrosa* no. 3 seed as received from the University of Nebraska with the pattern of seeds of the next generation grown at this location (Fig. 6g, h). The patterns were identical; this finding was reconfirmed when other samples were increased here and compared with the original material.

The patterns of total proteins extracted from four different accessions of *Ae. searsii*, which has been proposed as the donor of the B genome to tetraploid wheats and *T. aestivum* ssp. *vulgare* (Feldman 1978), are shown in Fig. 6 (a through d). The densitometric scan of the pattern of accession no. 1 is shown in Fig. 9e. *Ae. searsii* patterns had distinctive common features that, in the aggregate, distinguished it from the other species examined, including *Ae. longissima* to which it is closely related (Feldman 1978). One distinctive feature in comparison with other diploid species was the presence of well-defined bands corresponding to proteins with MW's in the range 100k-110k.

The most intense band of the *searsii* patterns corresponded to a MW of about 43k, which falls in the range of A3, and in this respect, the quantitative aspects of *searsii* patterns (Fig. 9e) were similar to those of the patterns of *speltoides* and *longissima* and differed from those of ssp. *boeoticum*, ssp. *monococcum*, *urartu*, and *squarrosa*, which had their intense bands in A4 (Fig. 9). For convenience in the discussion to follow, we shall refer to the quantitative distribution of proteins (according to MW) that is characteristic of ssp. *boeoticum*, ssp. *monococcum*, *urartu*, and *squarrosa* as a Type X pattern (most intense bands in A4) and that of *speltoides*, *longissima*, and *searsii* as a Type Y pattern (most intense bands in A3).

As can be seen from study of our SDS-PAGE patterns, most diploid species of *Triticum* and *Aegilops* have distinctive qualitative and quantitative characteristics to their patterns, which, when these characteristics are taken together, permit them to be distinguished from one another. A possible exception is the comparison of *T. monococcum* ssp. *boeoticum* and *T. monococcum* ssp. *monococcum* with *T. urartu*, but even these species probably can be distinguished from one another on the basis of their albumin proteins in A5.

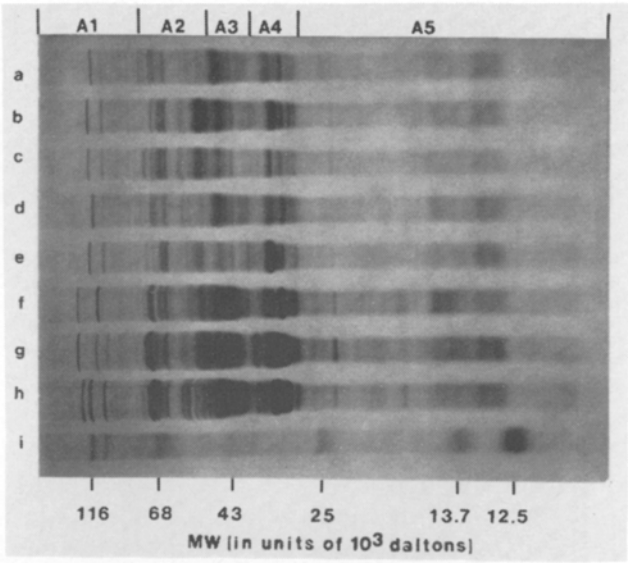

Fig. 7a-i. SDS-PAGE patterns of total proteins extracted from tetraploid species and accessions: **a** *T. turgidum* ssp. *carthlicum* no. 2; **b** *T. turgidum* ssp. *turgidum* conv. *durum* no. 3 ('Montferrier'); **c** *T. turgidum* ssp. *turgidum* conv. *durum* no. 2 ('Agathe'); **d** *T. turgidum* ssp. *carthlicum* no. 1; **e** *T. turgidum* ssp. *turgidum* conv. *polonicum* no. 1; **f** *T. turgidum* ssp. *dicoccoides* no. 2; **g** *T. turgidum* ssp. *dicoccoides* no. 3; **h** *T. turgidum* ssp. *dicoccoides* no. 4; **i** standard proteins. Migration from left to right

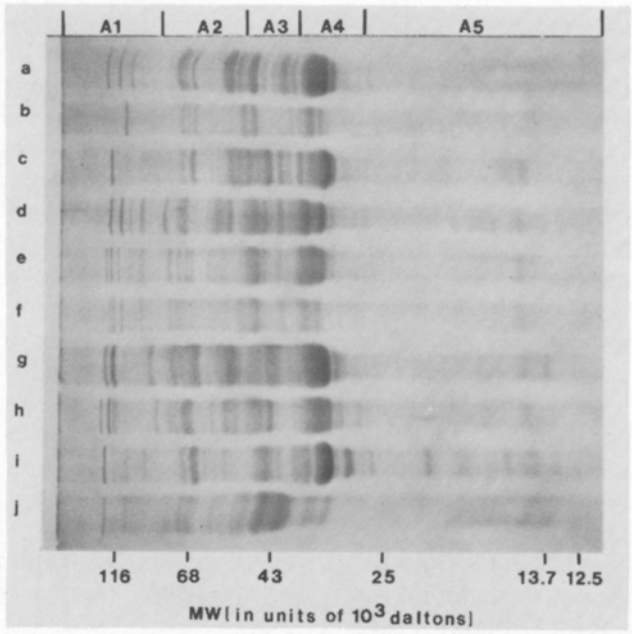

Fig. 8a-j. SDS-PAGE patterns of total proteins extracted from various species: **a** *T. turgidum* ssp. *dicoccoides* no. 1; **b** *T. turgidum* ssp. *turgidum* conv. *durum* no. 1 ('Leeds'); **c** *T. urartu* no. 2; **d** *T. aestivum* ssp. *vulgare* no. 4 ('Chinese Spring'); **e** *T. aestivum* ssp. *spelta* no. 1; **f** *T. aestivum* ssp. *vulgare* no. 1 ('Scout 66'); **g** *T. zhukovskyi* no. 1; **h** *T. timopheevi* ssp. *armeniacum* no. 1; **i** *T. monococcum* ssp. *boeoticum* no. 4; **j** *Ae. speltoides* no. 3. Migration from left to right

Tetraploid Species: Analysis of Patterns

Before discussing the relationships of tetraploid species to one another and to diploid species, we wish to consider the composite densitometric scanning patterns of Fig. 10a and b, which represent hypothetical amphiploids of *T. monococcum* ssp. *boeoticum* with *Ae. squarrosa* (Fig. 10a) and *T. monococcum* ssp. *boeoticum* with *Ae. speltoides* (Fig. 10b). Note that the former combination results in a Type X (or XX) pattern (most intense bands in A4) whereas the latter results in a combination of the Type Y (most intense bands in A3) and Type X patterns with

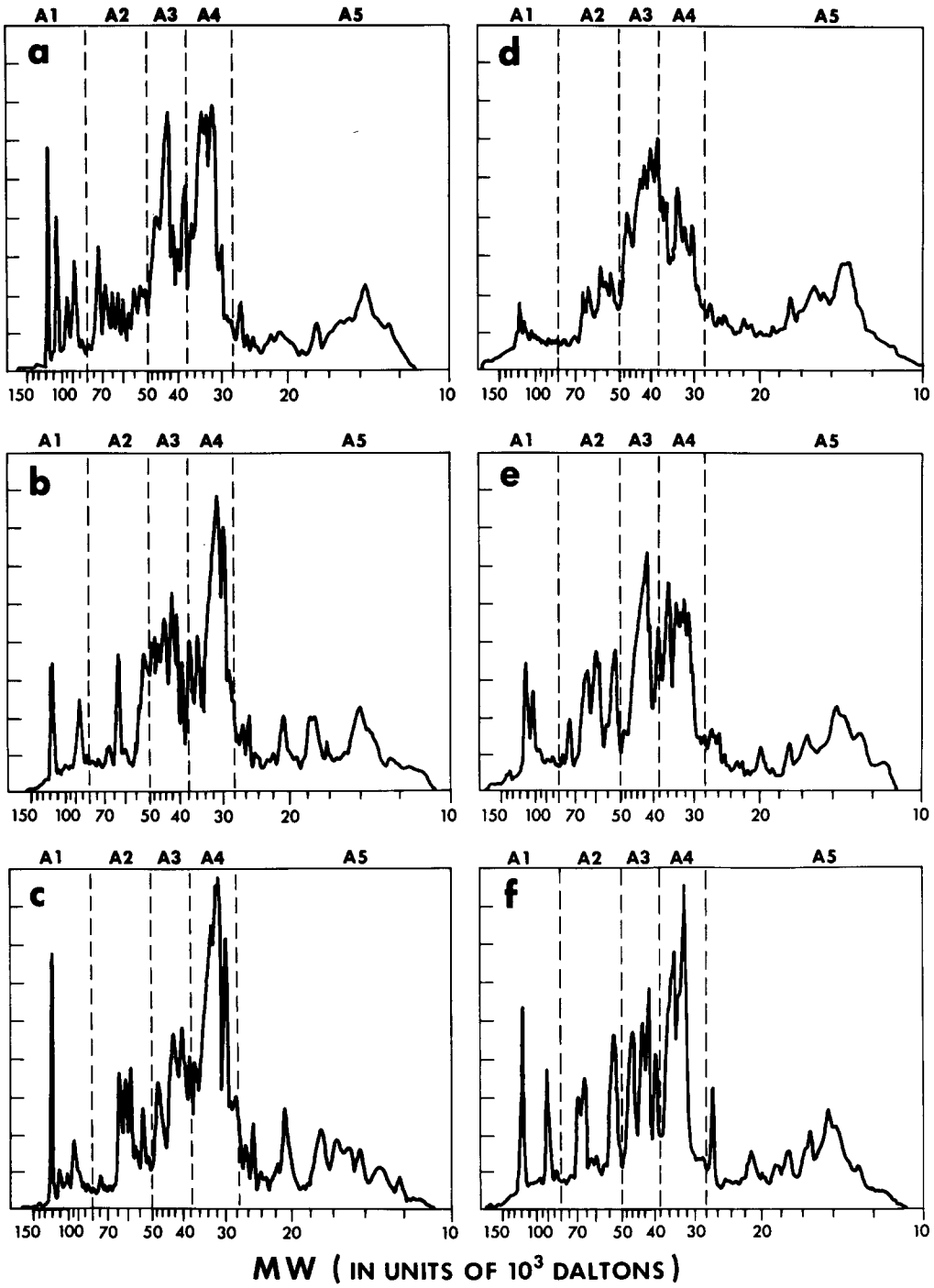

Fig. 9a-f. Densitometric scanning patterns of SDS-PAGE patterns (all from Fig. 3) of total proteins extracted from various species: a *T. aestivum* ssp. *vulgare* no. 1 ('Scout 66'); b *T. urartu* no. 1; c *T. monococcum* ssp. *boeoticum* no. 2; d *Ae. speltoides* no. 2; e *Ae. searsii* no. 1; f *Ae. squarrosa* no. 3

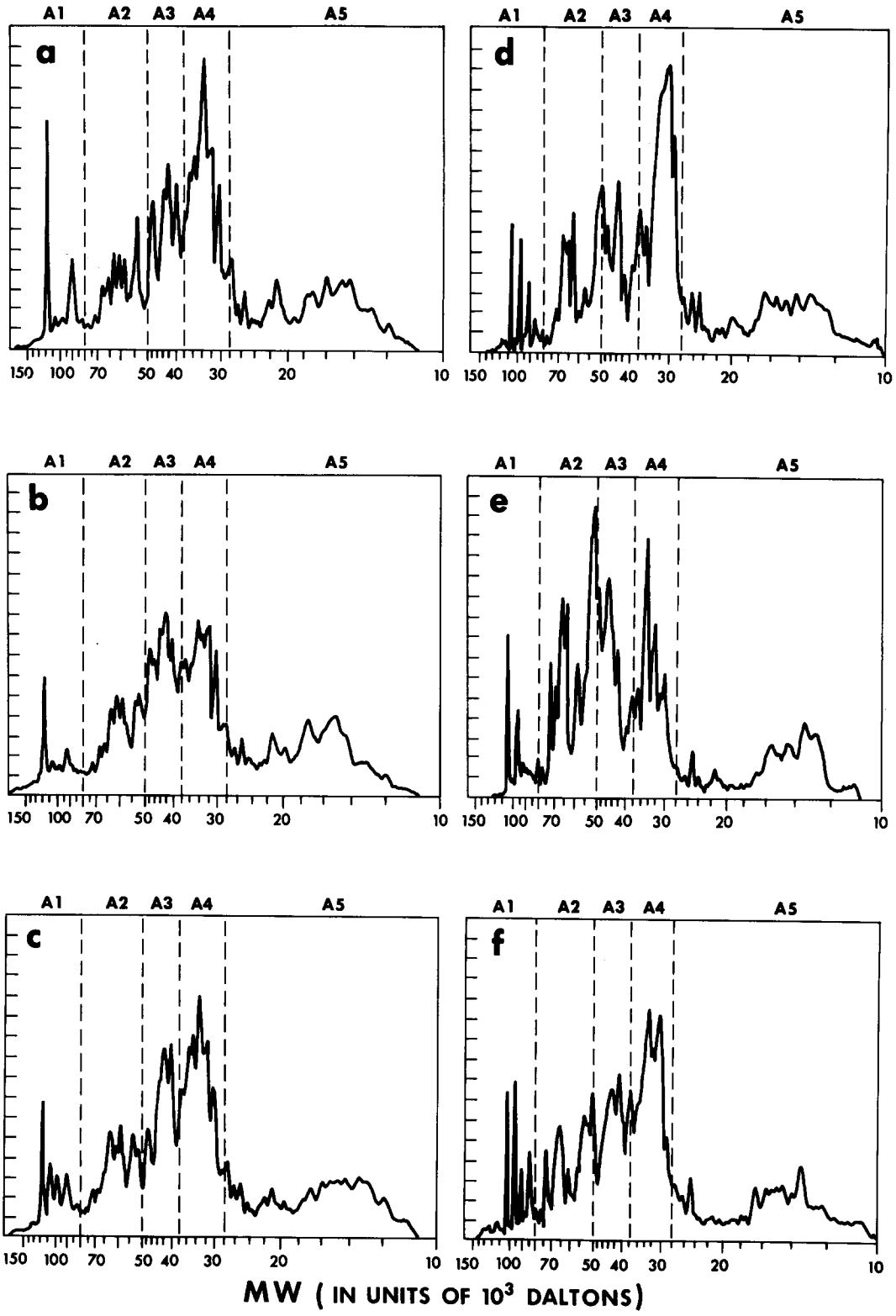

Fig. 10a-f. Densitometric scanning patterns of SDS-PAGE patterns of various polyploid species or combinations of diploid species to represent hypothetical amphiploid species: a composite pattern combining the patterns of *T. monococcum* ssp. *boeoticum* no. 2 and *Ae. squarrosa* no. 3 (both patterns of Fig. 3); b composite pattern combining patterns of *T. monococcum* ssp. *monococcum* and *Ae. speltoides* no. 2 (both patterns of Fig. 3); c composite pattern combining the patterns of *T. monococcum* ssp. *boeoticum* no. 2, *Ae searsii* no. 1, and *Ae. squarrosa* no. 3 (all patterns of Fig. 3); d *T. turgidum* ssp. *dicoccoides* no. 1 (pattern of Fig. 8); e *T. turgidum* ssp. *turgidum* conv. *durum* no. 2 ('Agathe'); f *T. aestivum* ssp. *vulgare* no. 4 ('Chinese Spring'), (pattern of Fig. 8)

major bands of about equal intensity appearing in both A3 and A4. We shall call this latter combination a Type XY pattern. Combination of the patterns of *T. monococcum* ssp. *boeoticum* with *T. urartu* (not shown) gave a Type XX pattern that was similar to the patterns of the parents. The combination of *T. monococcum* ssp. *boeoticum* and *Ae. searsii* (not shown) gave a Type XY pattern similar to that of the ssp. *boeoticum-speltoides* combination. On this basis, we might expect that if the genomes of tetraploid species of *Triticum* resulted from a simple summation of diploid genomes, only three types would be possible, Type XX, Type YY, or Type XY.

Actually, we found that the densitometric scans of the patterns of tetraploid species in our study ranged from Type X to Type Y in such a way as to indicate more than three possibilities. We have not included scans of all patterns, but an example of a Type X tetraploid pattern is shown in Fig. 10d (*T. turgidum* ssp. *dicoccoides* no. 1) whereas an example of a tetraploid pattern that approaches Type Y is shown in Fig. 10e (*T. turgidum* ssp. *turgidum* conv. *durum* no. 2). Because of the limited number of samples in our study and because of the small amount of variation in pattern for different species that have basically the same pattern type (compare Fig. 9b, c), we have not attempted a quantitative treatment of the variation from Type X to Type Y patterns. Accordingly, we cannot rule out continuous variation, but visual examination of our patterns indicated to us that the variation could be accounted for reasonably well by allowing for two loci per diploid genome that determine quantitative character and segregate independently. This extends the number of quantitative pattern types to five – Type XXXX, Type XXXY, Type XXYY, Type XYYY, and Type YYYY. Here, we assume that segregation has led to established lines that are homozygous so the triploid nature of endosperm does not need to be considered.

In support of this approach, we offer the following considerations:

(a) The genes coding for gliadin proteins (which make the most important contribution to our SDS-PAGE patterns) are clustered on two chromosomes of each genome (homoeologous groups 1, 6) in *T. aestivum* ssp. *vulgare* (Shepherd 1968, 1973; Wrigley and Shepherd 1973; Mitrofanova 1976; Kasarda et al. 1976b). This arrangement of genes presumably reflects the situation in diploid species of *Triticum* and *Aegilops* although it has been demonstrated only for *Ae. umbellulata* (Shepherd 1973). It is not known how the relative distribution of protein components is determined at the gene level during protein synthesis in the developing endosperm of cereal grains; it might be determined, for example, by the number of gene copies coding for particular components or by promoter sequences that control transcription frequency (Mecham et al. 1978). In any case, it seems likely that the factor or factors determining the quantity of protein synthesized from clusters of genes coding for gliadin components (and possibly other storage protein components) will be associated closely enough with the clusters to segregate with the clusters in an interchange of genetic material.

As an example of how multiple gene copies might affect protein distribution, if the predominance of protein corresponding in MW to A4 in *urartu* resulted from a greater number of genes (some of which might be identical copies) clustered on chromosome 6 relative to the number of copies clustered on chromosome 1 whereas if the predominance of protein corresponding in MW to A3 in *speltoides* resulted from a greater number of gene copies clustered on chromosome 1 relative to the number of copies clustered on chromosome 6, these characters might segregate independently. As an approximation, this would be true even if each chromosome had more than one cluster of genes coding for gliadin proteins as long as the clusters were reasonably closely linked. If interchange and segregation of genetic material could occur during polyploid formation, there would appear to be two loci affecting relative distribution of protein according to MW.

(b) The mechanism by which the natural tetraploids (and hexaploids) of *Triticum* were formed is not known, but formation may have involved combination of unreduced and reduced gametes to produce a spontaneous triploid followed by a similar process in the next generation to produce a tetraploid (Harlan and deWet 1975; Vardi 1973). In this mechanism, interchange and segregation of genetic material might occur during synapsis of partially homologous chromosomes of different species. Once polyploids have been established, interchange of genetic material between different lines or groups at the same level of ploidy is facilitated (Zohary and Feldman 1962; Harlan and deWet 1975).

Consideration (b) supports the possibility that segregation of genetic material may have occurred in conjunction with polyploid formation, but it is also possible that relatively few segregants (maybe only one) led to established polyploid lines (Harlan and deWet 1975). Consideration (a) provides support for the quantitative distribution of proteins according to MW being determined by more than one locus per diploid genome and we consider that an assumption of two loci per genome provides a reasonable approximation. We do not rule out the possibility of there being more than two loci controlling this distribution.

The Type X pattern we observed for *T. turgidum* ssp. *dicoccoides* no. 1 (Fig. 10d) would be Type XXXX in our modified classification. *T. turgidum* ssp. *turgidum* conv. *polonicum* no. 1 (densitometric scan not shown) was also Type XXXX. Apparently, all of the genes determining relative distribution of protein components according to MW in these two tetraploid accessions have been derived entirely from Type X (or Type XX in our

modified classification) diploid species. The most likely candidates for having contributed these genes are *T. monococcum* ssp. *boeoticum*, *T. urartu*, and *Ae. squarrosa*.

Although *Ae. speltoides* and *Ae. longissima* seem to have been the most favored candidates for contributor of the B genome to *T. turgidum* and *T. aestivum* (Jaaska 1978; Konarev et al. 1979), Johnson (1975) has proposed on the basis of morphological and other characteristics that the A genome of *T. turgidum* was contributed by *T. monococcum* ssp. *boeoticum* and that the B genome of this tetraploid was contributed by *T. urartu*. Also, Maan (1975) has suggested that *Ae. squarrosa* was involved in the origin of the cytoplasm of the tetraploid wheats and may have contributed genetic material to them. Our results on the quantitative distribution of proteins according to MW in these tetraploids indicate that either or both of the proposals of Johnson (1975) and Maan (1975) may be correct to some degree.

Caldwell and Kasarda (1978) noted that PAGE patterns of albumins and globulins (aluminum lactate buffer, pH 3.2, migration due mainly to differences in charge) were closely similar for the tetraploid *T. turgidum* ssp. *dicoccoides* and the hexaploid *T. aestivum* ssp. *vulgare*. Because the additional genome in the hexaploid was contributed by *Ae. squarrosa* (Konzak 1977), a likely explanation of the similarity of the patterns of proteins extracted from the two species is that albumins and globulins characteristic of *squarrosa* were already present in the tetraploid so that addition of the D genome from *squarrosa* effected little change in the pattern of the albumins and globulins. This result provides support for a possible contribution of genetic material from *squarrosa* to the tetraploid *T. turgidum*. It is possible, however, that a rather minimal contribution of genetic material could be sufficient to affect the synthesis of a few protein components.

The patterns of ssp. *dicoccoides* numbers 2 and 3 (Israel) and no. 4 (Lebanon) differed somewhat in their quantitative (and qualitative) aspects from ssp. *dicoccoides* no. 1 (Turkey). The accessions from Israel and Lebanon had densitometric scans (not shown) of Type XXXY in which the peaks of A4 were slightly more intense than the peaks of A3. Qualitatively, the SDS-PAGE patterns of these accessions (Fig. 7f-h) were similar in important respects to those of *longissima* (Fig. 5a-e) – especially in area A3 (MW's 38k-50k). Also, the two accessions from Israel (Fig. 7f, g) had high MW bands in their patterns corresponding to MW's of about 133k and 110k, which we have noted only in accessions of *longissima*. On the basis of these patterns, it seems that *longissima* has contributed genetic material to these accessions of ssp. *dicoccoides* from Israel.

The cultivated tetraploids species we examined showed almost the full range of densitometric scanning pattern types. As mentioned earlier, *T. turgidum* ssp. *turgidum* conv. *polonicum* no. 1 (scan not shown; gel pattern shown in Fig. 7e) had clearly a Type XXXX pattern with a single predominant peak in A4. *T. turgidum* ssp. *turgidum* conv. *durum* no. 2 cv. 'Agathe' (Fig. 10e) had a pattern that we somewhat arbitrarily assigned to Type XYYY, but which approached a Type YYYY pattern. Compare, for example, the scan of *Ae. searsii* in Fig. 9e with that of 'Agathe' in Fig. 10e. Durum cv. 'Montferrier' (Fig. 7b; scan not shown) had a type XXYY pattern with about equal intensities in A3 and A4 whereas durum cv. 'Leeds' (Fig. 8b; scan not shown) had a Type XXXY pattern with slightly greater intensities in A4 than in A3. *T. turgidum* ssp. *carthlicum* no. 1 and ssp. *carthlicum* no. 2 had nearly identical qualitative patterns (Fig. 7a, d) and scans (not shown) of Type XYYY with a slight predominance of peaks in A3 over those of A4. *T. turgidum* ssp. *dicoccum* no. 1 ('Khapli emmer') had a scanning pattern that was Type XXXY. The densitometric scan is not shown, but relative band intensities may be compared for areas A3 and A4 in the patterns of gliadins and total proteins from this accession in Figs. 2d and 3g, respectively.

The SDS-PAGE patterns of *T. timopheevi* ssp. *armeniacum* (*T. araraticum*) no. 1, *T. monococcum* ssp. *boeoticum* no. 4, and *Ae. speltoides* no. 3 are compared in Fig. 8h-j. The latter two species have been proposed as donors of the two genomes of the former species (Konarev et al. 1979). Our patterns of proteins from ssp. *boeoticum* no. 4 and *speltoides* no. 3 did not add especially well to yield the pattern of ssp. *armeniacum* no. 1; possibly other accessions might have provided a better fit. The pattern of our one accession of ssp. *armeniacum* was more complex in area A1 (MW's near 120k) than most tetraploids; this may indicate that more than two diploid species have contributed to the genetic makeup of this tetraploid. The densitometric scan (not shown) of ssp. *armeniacum* was Type XXXY with greater pattern intensities in A4 than in A3; this would be consistent with contributions to the genetic makeup of both genomes of this tetraploid from diploid species having type XX patterns.

Feldman (1978) proposed *Ae. searsii* as the likely donor of the B genome to polyploid wheats. Although it is difficult to draw any firm conclusion on this matter from our work, we have found that *searsii* does have moderately distinctive differences in SDS-PAGE protein pattern from *longissima*, to which it is closely related and that the tetraploids we have examined sometimes favor one or the other in their patterns. For example, the pattern of *searsii* no. 1 in Fig. 3i shows considerable similarity to that of *T. turgidum* ssp. *dicoccum* no. 1 (Khapli emmer) (Fig. 3g) whereas *T. turgidum* ssp. *dicoccoides* numbers 2 and 3 from Israel (Fig. 7f, g) show considerable similarity in their patterns to *longissima* (Fig. 5a-e).

On the basis of the qualitative and quantitative aspects of our SDS-PAGE patterns, we conclude that tetraploid species of *Triticum* are complex in their genetic makeup, that most accessions or cultivars will include in their genomes contributions from more than two diploid species, and that the amount of contribution from any given diploid species may vary among different accessions or cultivars of a tetraploid species.

Hexaploid Species: Analysis of Patterns

The SDS-PAGE patterns of proteins extracted from hexaploid wheats were generally more complex than those of proteins from diploid and tetraploid species – as would be expected. This was most noticeable in the range corresponding to MW's of 80k and higher (A1). The pattern of *T. aestivum* ssp. *spelta* no. 1 (Fig. 8e) and *T. aestivum* ssp. *vulgare* no. 1 (Fig. 8f) cv. 'Scout 66' were quite similar in their patterns. The other cultivars of ssp. *vulgare* that we examined ('Chinese Spring', Fig. 8d; 'Justin' and 'Atlas 66', patterns not shown) varied somewhat more in their patterns from that of 'Scout 66', but all these bread wheat cultivars had strong similarities in their patterns. Bread wheats may have had a monophyletic origin resulting from a cross between some particular cultivated tetraploid and *Ae. squarrosa* growing as a weed in a cultivated field (Kihara 1975); this would have occurred 6,000 to 8,000 years ago after the beginnings of agriculture about 10,000 years ago. This possibility is supported by the similarity of the pattern of 'Chinese Spring' (Fig. 8d) and other cultivars of ssp. *vulgare* insofar as Chinese cultivars may have been separated from European cultivars for several thousand years (Zurabishvili et al. 1978).

The patterns of bread wheat cultivars are not perfectly identical, however, and if they have derived from a single cross, they have become modified somewhat over the years through introgression. The densitometric scans of the SDS-PAGE patterns of cvs. 'Scout 66' and 'Chinese Spring' may be compared in Fig. 9a and 10f, respectively, and with our synthetic composite of *T. monococcum* ssp. *boeoticum*, *Ae. searsii*, and *Ae. squarrosa* in Fig. 10c. The patterns of 'Chinese Spring' and 'Atlas 66' (not shown) were of Type XXXXYY as might be expected if a tetraploid wheat with a pattern of Type XXYY combined with *squarrosa* (Type XX) in a simply additive fashion. The scans of protein patterns of 'Scout 66' (Fig. 9a) and 'Justin' (not shown) were similar to one another. Both of these cultivars had patterns with a moderately intense peak in A3 corresponding to a MW of about 45k so that their patterns might be considered Type XXXYYY; these wheats seem to have a strong contribution from one or more Type YY diploids. This type of pattern might result also from a displacement of components contributed to A4 by one genome relative to the position of components contributed to A4 by other genomes. This could diminish the reinforcement of bands in the A4 part of the pattern resulting from overlapping components.

There was considerable similarity between the SDS-PAGE patterns of proteins extracted from *T. timopheevi* ssp. *armeniacum* no. 1 (Fig. 8h) and from *T. zhukovskyi* no. 1 (Fig. 8g) as would be expected if the hexaploid *zhukovskyi* resulted from addition of a second A genome to the AB genome of ssp. *armeniacum* (Konarev et al. 1979). The albumins and globulins of A5 did not show more than moderate coincidence, however, when these two species were compared. The type of *T. monococcum* var. 'boeoticum' that contributed importantly to the AB genome of ssp. *armeniacum* may have differed – at least in the pattern of its albumins and globulins – from the type or types that contributed importantly to the two A genomes of the hexaploid *zhukovskyi*.

The densitometric scan of the pattern of proteins from *zhukovskyi* was notable for having extremely high intensity in A4. This would be consistent with a simple addition of a diploid genome with Type XX pattern (such as *T. monococcum* ssp. *boeoticum*) to the tetraploid genome with Type XXXY pattern, as we found for *T. timopheevi* ssp. *armeniacum*, to yield a hexaploid with Type XXXXXY pattern. (Our visual assignments of patterns to types would not discriminate such a pattern from a Type XXXXXX pattern).

Speciation and Protein Expression

We have little understanding yet of the genetic control of storage protein synthesis in cereal grains, but on the basis of our finding that the MW of major gliadin (or prolamin) components is species specific to a considerable degree, we speculate that this difference came to exist during speciation of the *Triticinae* and may have some fundamental connection with this process. These MW differences are notable in consideration of the finding of Autran et al. (1979) that the N-terminal amino acid sequences show little difference for prolamins of different species of *Triticum* and *Aegilops*; about 70-80% of the prolamins of these species belong to the 'α-type' or the 'γ-type' on the basis of N-terminal sequences with both types present in all the different species examined.

In consideration of the large amounts of repetitive DNA in the genomes of higher plants and the variation in these amounts among different species, Thompson and Murray (1979) proposed that amplifications of portions of the DNA, transpositions of short pieces of DNA, and deletions of portions of the DNA have been frequent evolutionary events. Genes coding for gliadin proteins may have undergone duplications followed by transpositions of portions of the gene DNA that resulted in modifications of the sizes of gene copies. Subsequently, modified genes may have again been duplicated. This last duplication could have occurred in conjunction with other events, such as extensive amplification of DNA as considered by Thompson and Murray (1979), leading to the formation of new species with differences that included differences in MW's of their major gliadin components.

Relations to Protein Quality

The highest-MW proteins of gluten and glutenin in bread wheats have been proposed from various evidence to con-

tribute importantly to protein quality in breadmaking (Khan and Bushuk 1979; Wall 1979; Payne et al. 1979); components contributed by the D genome may be of special importance (see Wall 1979 for discussion). Our results do not provide information about likely relationships of these high-MW components to quality, but we note that the components with the highest MW's in bread wheats (MW's close to about 120k) have equivalents in *T. monococcum* ssp. *boeoticum*, *T. urartu*, and *Ae. squarrosa*. It seems that these components have been contributed by the A genome as well as the D genome.

We have found that the two bread wheat cultivars in our study that have good baking quality, 'Scout 66' and 'Justin', also had important peaks in their SDS-PAGE patterns that correspond to proteins with MW's near 45k. The two cultivars that did not have good baking quality, 'Chinese Spring' and 'Atlas 66', showed much less protein corresponding to this MW. Because this peak may correspond to the ethanol-soluble glutenins and high-MW gliadins (Bietz and Wall 1973) and because the proteins of this peak are present in relatively large amounts in gluten and glutenin (Kasarda et al. 1976a), a possible relation between the amounts of these proteins and mixing and baking characteristics of bread wheat cultivars should be investigated.

In contrast, the highest-MW proteins found in the endosperm of bread wheats are not present in relatively large amounts (probably about 10% of the total protein); the apparent areas corresponding to these proteins in our densitometric scanning patterns of Fig. 9 and 10 overestimate their true proportions. This artifact results because the bands of the pattern are so sharp that they give an intense absorption, but they do not occupy much of the total area – most of the area corresponding to these bands in the graphical representations of the scans is taken up by the line that defines them. Of course, it is possible that even relatively small amounts of these high-MW proteins may have strong effects on dough quality, but this had not been demonstrated.

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Reference to a company and/or product named by the Department is only for purposes of information and does not not imply approval or recommendation of the company or product to the exclusion of others that may also be suitable.

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