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Assessment of Genomic and Species Relationships in *Triticum* and *Aegilops* by PAGE and by Differential Staining of Seed Albumins and Globulins

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Summary. Endosperm protein components from common bread wheats (*Triticum aestivum* L.) and related species were extracted with aluminum lactate, pH 3.2, and examined by electrophoresis in the same buffer. Electrophoretic patterns of the albumins and globulins were compared to evaluate the possibility that a particular species might have contributed its genome to tetraploid or hexaploid wheat. Together with protein component mobilities, differential band staining with Coomassie Brilliant Blue R250 was employed to test the identity or non-identity of bands. Eight species and 63 accessions, representative of *Triticum* and *Aegilops* were tested. Considerable intraspecific variation was observed for patterns of diploid but not for tetraploid or hexaploid species. Patterns of some accessions of *Triticum urartu* agreed closely with major parts of the patterns of *Triticum dicoccoides* and *T. aestivum*. A fast-moving, green band was found in all accessions of *T. urartu* and of *Triticum boeoticum*, however, that was not found in those of *T. dicoccoides* or *T. aestivum*. This band was present in all accessions of *Triticum araraticum* and *Triticum zhukovskyi*. Patterns of *Aegilops longissima*, which has been suggested as the donor of the *B* genome, differed substantially from those of *T. dicoccoides* and *T. aestivum*. Finally, two marker proteins of intermediate mobility were also observed and may be used to discriminate between accessions of *T. araraticum/T. zhukovskyi* and those of *T. dicoccoides/T. aestivum*.

Key words: Wheat proteins – Aluminum lactate extraction – Genome donors – Wheat evolution

Introduction

Common bread wheats (*Triticum aestivum* L.) belong to a hexaploid species (*AABBDD*) for which the diploid progenitors were probably forms of *Triticum* and *Aegilops*. In polyploids of the *aestivum* type, the *D* genome is well-established as some form of *Ae. squarrosa* (Kihara 1944; McFadden and Sears 1944; Johnson 1972). The *A* genome is more tentatively assigned to *T. boeoticum* (Morris and Sears 1967; Johnson 1973; Johnson 1975), but the identity of the *B* genome is still the subject of considerable controversy (Sears 1956; Riley et al. 1958; Johnson 1975; Sarkar and Stebbins 1956; Chapman et al. 1976; Konarev et al. 1976; Vittozzi and Silano 1976).

Much less disagreement exists concerning the origin of tetraploid wheats of the *timopheevi* type, for which an initial interspecific cross between *T. boeoticum* and *Aegilops speltoides* has been postulated (Johnson 1975; Konarev et al. 1976). Similarly, the hexaploid species *Triticum zhukovskyi* probably resulted from a second hybridization of *T. timopheevi* with *T. boeoticum* followed by chromosome doubling to form a new, fertile species (Johnson 1975; Konarev et al. 1976).

Hall, Johnson and coworkers (Hall 1959; Hall et al. 1963; Johnson and Hall 1965; Johnson et al. 1967) pioneered in the use of electrophoretic patterns of seed proteins to evaluate possible contributors of the *A* and *B* genomes to emmer, *timopheevi*, and *aestivum* types. But the solvent used was 70% ethanol, which extracts only some of the albumin and globulin components. In the present work, we describe an investigation of the electrophoretic mobility of aluminum lactate-soluble proteins from the endosperm of common bread wheats and related species. As with aqueous ethanol, aluminum lactate also extracts the full range of prolamine components. But we shall place emphasis on the albumins and globulins because prolamine patterns showed even greater intraspecific variation than did the faster-moving components. The genetic complexity of prolamine fractions has been reported previously (Elton and Ewart 1962; Lee and Ronalds 1967; Shepherd 1968; Wrigley and Shepherd 1974; Konarev et al. 1976). According to those reports, faster-moving components are genome-specific whereas prolamines contain

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specific proteins which denote not only genome but also genus, species, and subspecies.

An important aspect of our research is the use of differential staining effects to assess the probable identity or non-identity of apparently homologous bands. This work was undertaken in an attempt to extend the usefulness of electrophoresis in genetic studies and to clarify some of the questions regarding the genome donors to tetraploid wheats.

Materials and Methods

Species examined

Species examined were as follows (the number of accessions is given in parentheses): *Aegilops speltoides* (7), *Aegilops longissima* (4), *Aegilops squarrosa* (3), *Aegilops sharonensis* (1), *Aegilops umbellulata* (1), *Aegilops uniaristata* (1), *Aegilops bicornis* (1), *Aegilops caudata* (1), *Aegilops comosa* (1), *Triticum boeoticum* (11), *Triticum monococcum* (4), *Triticum urartu* (11), *Triticum dicoccoides* (6), *Triticum araraticum* (5), *Triticum aestivum* (5), and *Triticum zhukovskyi* (1). Details of the accessions used for the electrophoretic patterns of the figures are given in the captions.

Methods

In preliminary experiments, partially purified samples of wheat gliadins, globulins or albumins were prepared. Albumins and globulins were obtained from flours of *T. durum* 'Mindum' and *T. aestivum* 'Scout' by extraction with 0.15M NaCl and fractional precipitation with ammonium sulfate, as described by Silano et al. (1969). The precipitated fraction (albumins) was re-dissolved in 0.15M NaCl, dialyzed against water and lyophilized. Mixed gliadins were obtained from undefatted flour by extraction with 70% ethanol. Purification was according to the procedure of Charbonnier (1970), modified as follows: the salt precipitated fraction was re-solubilized in and dialyzed against 0.01M acetic acid; the preparation was then dialyzed against water and lyophilized. Protein solutions were prepared by dissolving each protein in 0.0085M aluminum lactate buffer, pH 3.2, at a concentration of 2 mg protein per ml. Buffer-soluble proteins were also extracted directly from 'Scout' flour by trituration and centrifugation as described below. Protein solutions were examined by electrophoresis to compare the components extracted in buffer to those of purified preparations and to test whether fractionation appreciably improved band resolution or intensity. The same components were obtained in buffer extracts as in partially purified samples and band resolution and intensity were also comparable. Therefore, subsequent experiments were conducted on total, buffer-extractable proteins.

Seed embryo and adjacent tissues were dissected from each kernel. The remainder was weighed (30 to 50 mg) and pulverized to a dry powder with a mortar and pestle; more than a single seed was required for most diploid species examined. Proteins were extracted with a 10X volume of buffer by thoroughly grinding the endosperm-buffer suspensions, which were then centrifuged for 20 min at 27,500 X g, 3°. Aliquots (25 to 40λ) of supernatant fractions were taken for immediate electrophoresis; the remainder of each extract was stored in a liquid nitrogen refrigerator until subsequent analysis. No differences in protein patterns were observed between fresh and stored samples. Good band resolution, adequate for visual inspection or photographic records, was obtained with 25 to 30λ of clarified extract. Larger aliquots (40λ) were used on gels photographed for journal reproduction. At the higher concentration weaker bands were more readily seen, but other bands were noticeably distorted or fused.

Electrophoresis was performed on 7% polyacrylamide gel-slabs (ratio of acrylamide to bis-acrylamide was 22:1; 23 X 12.5 X 0.5 cm) in 0.0085M aluminum lactate, pH 3.2. (Aluminum lactate was obtained from ICN Biochemicals, K & K Division, and was decolorized with activated charcoal before use.) Gels were cast in water and soaked overnight in aluminum lactate buffer; the same solution was subsequently used as electrophoresis buffer in the horizontal gel equipment. One sample of *T. aestivum* 'Cheyenne' was included on most gels as a reference. Separations were conducted for 5 hrs at a voltage differential of 8.2 V/cm, which caused the purothionin bands to run off the end of the gel but gave better resolution of the remaining bands. Therefore, the leading band in each pattern was the fastest-moving albumin band.

Protein bands were fixed and stained for 2 to 5 days with a 0.02% solution of Coomassie Brilliant Blue R250. The dye was dissolved in absolute ethanol and diluted with aqueous trichloroacetic acid to final concentrations of 5% ethanol and 6% TCA. At these concentrations, background stain was minimal and de-staining was unnecessary. But occasionally, gels were soaked in water for 1 or 2 days to improve the clarity of some colors. To enhance differential color effects, gels were studied or photographed through an orange filter, Wratten G 15.

Band migration is expressed as relative mobility by comparison with the leading Cheyenne band (R_c). Reported mobilities were verified by running the same sample at a different position on the gel and the error in R_c was ± 0.01. Patterns representative of most groups studied were selected for presentation in this paper.

Results

Differential Dye Binding of Wheat Protein Fractions

Coomassie Brilliant Blue R250 stains gliadin components a deep indigo; the soluble protein components stain shades of blue or purple. When protein bands were viewed through an orange filter, however, the gliadins appeared deep brown, whereas albumins and globulins appeared various shades of brown or green. Minor differences were noted from gel to gel but the different shades or intensities of green or brown seemed to be characteristic of a particular protein component, although band density may affect the perception of its shade. The procedure was especially helpful for testing the probable identity or non-identity of similarly-migrating components on the same gel and for locating marker protein bands. In some cases it was possible to discriminate between apparently identical bands because of distinct color differences. In other cases, a band sometimes appeared to be a single entity based on electrophoretic mobility, whereas staining characteristics suggested the superposition of two components having slightly different chemical nature. This was confirmed by resolution of such bands in exceptionally good experi-

ments. All further references to band color will denote color as seen through the filter.

Electrophoretic Profiles and Dye-Binding Characteristics of the Diploids Examined

In general, albumin and globulin fractions migrated in the region 0.40 to 1.04. Common features and variations in protein patterns for accessions of *T. boeoticum* and *T. urartu* and for species of *Aegilops* are illustrated in figures 1, 2, and 3, respectively. No band could be associated unambiguously with a specific diploid species based on mobility alone. But a combination of component migration and band color revealed notable differences. For convenience in presenting results, albumin-globulin patterns were divided into three regions of mobility.

(a) Fast-Moving Components, R_c 0.90 to 1.04: Four bands were observed in this region, including the reference band. These merit attention for both their frequency of occurrence and density of color. Most evident was a leading green component of R_c 1.03. This somewhat diffuse band was present in each accession of *T. boeoticum* (Fig. 1) and *T. urartu* (Fig. 2) but was found in only one-third the species of *Aegilops* tested. The ultimate band was usually followed by a discrete, brown band or doublet at 0.95 to 0.98 in accessions of *T. urartu* or *T. boeoticum*. In at least one accesion of *T. urartu*, however, this brown band appeared to be diffuse rather than discrete (Fig. 2B). In the species of *Aegilops* tested, components of R_c 0.95 to 0.98 appeared either as a discrete brown band, a diffuse green-brown band or were entirely absent (Fig. 3). The third component in this region stained green-brown and was a diffuse band located at about 0.93. It occurred strongly in about one-third of the *Aegilops*, was weakly present in most accessions of *T. boeoticum* but was entirely absent from accessions of *T. urartu*.

(b) Intermediate Components, R_c 0.60 to 0.90: In general, 7 to 13 bands were observed here. All species of *Aegilops* showed differences in their electrophoretic patterns that are probably species-related. In most cases the differences were substantial (see patterns of *Ae. longissima*, *Ae. sharonensis* and *Ae. uniaristata* in Figs. 3C, E and F). Rather than detail all such differences, a few patterns of interest are shown in Fig. 3 but most aspects of the patterns will

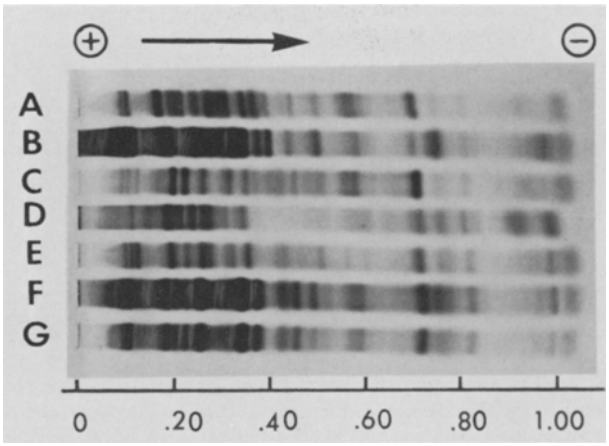

Fig. 2. Gel electrophoretic patterns of seed proteins extracted from *T. urartu* compared with *T. aestivum* 'Cheyenne'. Accessions: A, G1844 (Turkey); B, G3135 (Lebanon); C, G1944 (Turkey); E, G1876 (Turkey); F, G2991 (SSR); G1753 (Transcaucasia). D, *T. aestivum* 'Cheyenne'

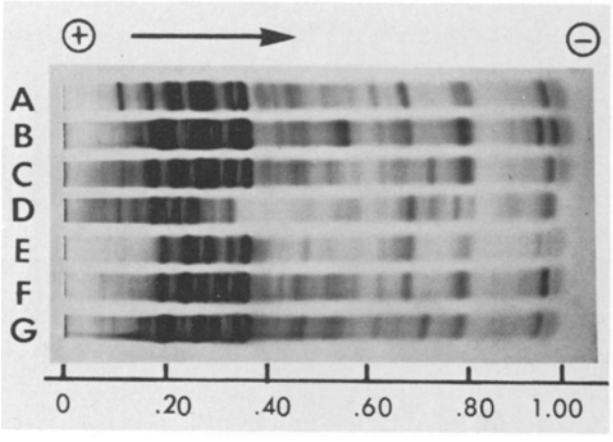

Fig. 1. Gel electrophoretic patterns of seed proteins extracted from *T. boeoticum* compared with *T. aestivum* 'Cheyenne'. Accessions: A, G2512 (Iran); B, G1758 (Transcaucasia); C, G1804 (Turkey); E, G1789 ssp Thaoudar (Turkey); F, G3116 (Lebanon); G, G1769 (Turkey). D, *T. aestivum* 'Cheyenne'

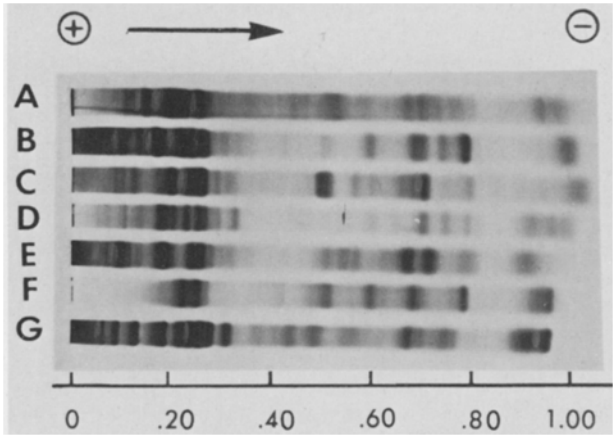

Fig. 3. Gel electrophoretic patterns of seed proteins extracted from *Aegilops* compared with *T. aestivum* 'Cheyenne'. Species of *Aegilops*: A, *speltoides* G617; B, *squarrosa* 64-1153; C, *longissima* G1304; E, *sharonensis* G614; F, *uniaristata* G1296; G, *umbellulata* G630. D, *T. aestivum* 'Cheyenne'

be discussed in connection with those of the polyploids (Figs. 4 and 5).

For accessions of *T. boeoticum* or *T. monococcum*, the entire pattern was distinguished by the somewhat regular character of band intervals (Fig. 1). (Patterns of accessions of *T. boeoticum* and those of *T. monococcum* did not differ appreciably so results of the latter will not be discussed separately). A pair of light brown bands distinguished this group from others. These two bands had essentially the same intensity, generally migrated at 0.70 and 0.83 and usually had no strong intervening bands between them. In some accessions, however, the slower-moving band of the pair appeared to be replaced by a band of somewhat higher mobility located at 0.76. Many accessions of *T. urartu* were characterized by a cluster of 3 to 5 dense bands in the region of 0.71 to 0.83 (Fig. 2E, F, and G): First, was a medium intensity brown band (0.83) followed by a medium intensity green band or doublet (0.76) and a deep brown band (0.72); the latter was frequently comparable in density to prolamine components. In other accessions of *T. urartu* (Fig. 2A, B. and C), the brown band (0.72) apparently was transposed to 0.75 (Fig. 2B) and other bands characteristic of the first group were missing or weak.

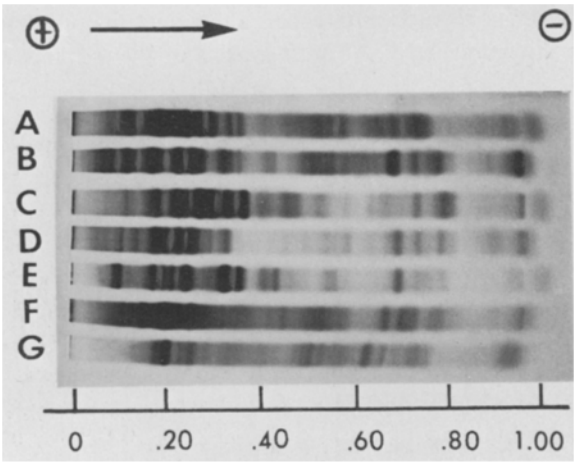

Fig. 4. Gel electrophoretic patterns of seed proteins extracted from diploid, tetraploid and hexaploid species compared with *T. aestivum* 'Cheyenne'. A, *T. zhukovskyi* G986; B, *T. dicoccoides* G3074; C, *T. boeoticum* ssp Thaoudar G1804 (Turkey); D, *T. aestivum* 'Cheyenne'; E, *T. urartu* G1753 (Transcaucasia); F, *Ae speltoides* G617; G, *Ae. speltoides* 68-349

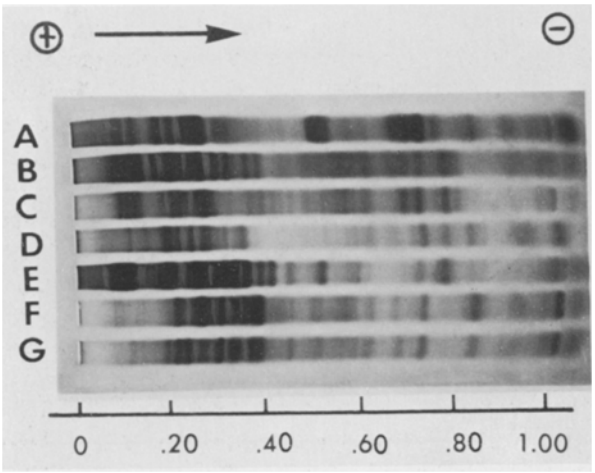

Fig. 5. Gel electrophoretic patterns of seed proteins extracted from diploid, tetraploid and hexaploid species compared with *T. zhukovskyi*. A, *Ae. longissima* G1304; B, *T. zhukovskyi* G986; C, *T. araraticum* G2541 (Iran); D, *T. aestivum* 'Cheyenne'; E, *T. urartu* G3135 (Lebanon); F, *T. boeoticum* Thaoudar G3116 (Lebanon); G, *T. monococcum* 64-1142

(c) Slow-Moving Components, R_c0.40 to 0.60: As had been observed for intermediate components, the patterns of *T. boeoticum* seemed to have a more regular character than those of other species. Many accessions of the *Triticum* diploids had a fairly sharp, brown band at 0.44 (*T. boeoticum*) or 0.46 (*T. urartu*); this brown band was frequently bracketed by green bands at about 0.40 and 0.47 (Fig. 1A, C, F, G; Fig. 2E, F, G). Species of *Aegilops* examined rarely showed dense banding in this region.

Camparison of Different Levels of Ploidy

Typical experiments demonstrating observed similarities and dissimilarities are shown in Figs. 4 and 5. Based on results such as those summarized in Figures 1, 2 and 3, diploid and tetraploid samples shown in Fig. 4 were selected for apparent nearness of fit to *T. aestivum* 'Cheyenne'; those in Fig. 5 were selected for apparent relatedness to *T. zhukovskyi.*

(a) *Triticum aestivum* and *Triticum dicoccoides*: Patterns of different varieties of *T. aestivum* were alike in the region corresponding to albumin and globulin components. Patterns of various accessions of *T. dicoccoides* sometimes differed from one another in minor ways but were similar and sometimes identical to those of some accessions of *T. aestivum.* Therefore, it would seem that the *D*-genome contributed few new bands to the tetraploid ancestor of *T. aestivum.* Notable differences of *T. aestivum* from some accessions of *T. dicoccoides* were the absence of both the fairly prominent green-brown band at 0.93 and the brown band at 0.80.

Relationships of diploid species to *T. dicoccoides* were more difficult to discern than those of *T. dicoccoides* to *T. aestivum.* No combination of patterns from two diploid species produced a close overall fit to patterns of the tetraploids.

In the region of fast-moving components, all accessions

of *T. boeoticum* and *T. urartu* had a fast green (or green-brown–depending upon resolution) band at 1.03 that was not present in accessions of *T. dicoccoides* or in *T. aestivum*. The band at 1.03 was also present in two of four accessions of *Ae. longissima*. Some accessions of *Ae. squarrosa* had a green band at 1.00 similar to that of *T. aestivum* and *T. dicoccoides* (Fig. 3B). A fast-moving green-brown band at 0.93 in *T. aestivum* and in some accessions of *T. dicoccoides* had a counterpart in *Ae. sharonensis*, *Ae. umbellulata*, *Ae. caudata*, *Ae. comosa*, *Ae. longissima*, and weakly, in accessions of *T. boeoticum*. This band was absent from all accessions of *Ae. speltoides* and was present in only one accession (0-623) of *Ae. squarrosa*.

In the intermediate region, patterns of *T. aestivum* consisted of a weak, brown band at 0.83 followed by a medium-intensity, brown band at 0.81; a green-brown band of medium intensity at 0.75; and an intense brown band at 0.71, apparently made up of two different-colored bands. This last band may have an underlying green component. The band at 0.71 in *T. dicoccoides* appeared the same as in *T. aestivum*, but in *T. urartu* it was generally a pure brown. *Ae. speltoides* and *Ae. sharonensis* had green bands near 0.71 in some accessions.

The green component of the intermediate band near 0.75 in the polyploids was present in most accessions of *T. urartu*; it was not present in any accession of *T. boeoticum* or *Ae. longissima* but some accessions of *Ae. speltoides* and *Ae. sharonensis* had green components of similar mobility. A fairly-pure-green band of mobility 0.75 was also present in accessions of *Ae. squarrosa* (Fig. 3B).

A pure-green band of mobility 0.65 was characteristic of *T. aestivum* and all accessions of *T. dicoccoides*; the equivalent of this band was found in one accession of *Ae. longissima* (7011-7YH, #4) and in one of *Ae. speltoides* (68-349). The green band at 0.65 was characteristic only of *T. dicoccoides* and *T. aestivum*; it did not appear in the patterns of *Triticum araraticum* or *Triticum zhukovskyi*.

The polyploids were essentially devoid of intensely-staining bands in the slow region (0.40 to 0.60). Although both *T. boeoticum* and *T. urartu* usually had green components in this range, such components were not present in *T. aestivum* and only one accession (G2107) of *T. dicoccoides* showed a slow-moving, green component (R_c 0.42). The species of *Aegilops* examined had no green bands of significant intensity in this region.

(b) *Triticum araraticum* and *Triticum zhukovskyi*: The pattern of *T. zhukovskyi* was nearly identical to the patterns of most accessions of *T. araraticum*, but patterns of these two species were distincly different from those of *T. aestivum* and *T. dicoccoides* (Fig. 4A, B, D, and Fig. 5B, C, D). *T. zhukovskyi* and *T. araraticum* were notable for the presence of a leading green band at 1.03 that also characterized *T. boeoticum* and *T. urartu*. This band was not present in *T. aestivum* or *T. dicoccoides*; instead, the latter species were characterized by a leading green band with a mobility of 1.00. *T. zhukovskyi* and *T. araraticum* lacked the brown band at 0.80 and the green band at 0.65 that were characteristic of *T. aestivum* and *T. dicoccoides*. A green band at 0.68 was present in *T. zhukovskyi* and all accessions of *T. araraticum*, but absent from *T. aestivum* or *T. dicoccoides*. A similar green or green-brown band was found in some accessions of *T. boeoticum* and some species of *Aegilops*.

Discussion

Hall and Johnson (1963) first applied electrophoretic separations as a biochemical approach to genome identification. They found that electrophoresis patterns of albumins extracted from seeds with 70% ethanol showed little intraspecific variation; therefore, such patterns could be used to distinguish different species. By assuming that protein patterns of the polyploids represented the sum of parental patterns, they analyzed the patterns of polyploid wheats and related species to evaluate probable genome donors to the polyploids. In contrast to that report, a commonly observed problem in utilizing PAGE for biochemical genetic studies is the variability for accessions within a species (Elton and Ewart 1962; Graham 1963; Boyd et al. 1969; Dronzek et al. 1970). Therefore, one aim of our research was to improve the sensitivity of the analytical procedure such that subtle or previously undetected differences might be revealed.

We have attempted to increase the information obtainable from electrophoretic analyses by using aluminum lactate buffer to extract proteins from the seed and as electrophoresis buffer; by improving the resolution obtained during electrophoresis; and by making use of differential staining effects in the analysis of patterns. The basis for these modifications is explained in the following paragraphs.

Extraction of seeds with aluminum lactate buffer solubilizes many more protein components than does extraction with 70% ethanol. In addition, the use of aluminum lactate as an electrophoresis buffer, according to our method, produces a relatively high resolution of components. Johnson and Hall (1965) used aluminum lactate buffer for electrophoresis in some early work, but Johnson (1967, 1972) subsequently switched to alanine-acetic acid buffer, pH 4.3. The patterns of Johnson and Hall (1965) were of a lower resolution than those presented in this paper.

H.S. Dhaliwal had pointed out to us that differential color effects could be seen when gel patterns were stained with Coomassie Brilliant Blue R250 and that the effect

was enhanced when patterns were viewed through an orange filter; some components appeared brown and others green. We thought this effect could aid in the discrimination of different protein components having closely similar electrophoretic mobilities. Differential staining effects had also been observed by Minetti et al. (1971, 1973); they found that certain preparations of aniline blue-black stained prolamines a reddish-brown color, whereas albumins and globulins were stained blue or greenish-black, respectively. The effect we observed differed in that it did not seem to be class-specific: We found an albumin preparation to contain both brown and green bands. Accordingly, by combining color and mobility in the analysis of patterns, we were able to associate green bands at R_c 1.03 and at R_c 0.68 with *T. araraticum* and *T. zhukovskyi* whereas *T. dicoccoides* and *T. aestivum* lacked such bands; the latter two species had comparable green bands with mobilities of 1.00 and 0.65. These bands were useful in distinguishing wheats of the *timopheevi-zhukovskyi* group from those of the emmer-*aestivum* group.

We also found a number of bands or groups of bands that were fairly characteristic of a given species; these enabled us to evaluate the most probable diploid donors to the polyploids. Because of intraspecific variability and because no protein band could be associated uniquely with a given species, however, we were unable to make unequivocal identifications of donors. Similar problems have arisen with most other approaches to the problem. Some reasons for this have been reviewed recently by Konarev et al. (1976): During the course of evolution, genomes may have changed following their incorporation into the polyploid; new genetic material may have been acquired after polyploid formation through interaction with related species and the true donors either may not have been collected or may be extinct.

Relationship of Diploids to Polyploids

Accessions of a given species collected from the same geographic area usually had similar electrophoretic patterns for their albumins and globulins, but these patterns often differed substantially from those of accessions collected from more distant geographical areas. For example, the patterns of several accessions of *T. boeoticum* from Turkey were nearly identical, but differed from the pattern of an accession from Lebanon in one important band; an accession from Transcaucasia differed in several major bands from both the Turkish and Lebanese accessions. This intra-specific variability contributed to the difficulty in making firm associations of polyploid genomes with particular diploid species.

Occasionaly, however, we found that a group of bands from some diploid accessions fit exceptionally well with an equivalent group in one or more of the polyploid species with regard to colors and mobilities. For example, patterns of *T. aestivum* and *T. dicoccoides* near 1.00 fit best with some accessions of *Ae. speltoides* (Fig. 4B, D, F) which had a combination of a diffuse, green band at 1.00 closely followed by a fairly discrete, brown band at 0.98 (sometimes unresolved) that were similar to those of the polyploids. In the intermediate region of the profiles, a cluster of bands (0.70 to 0.85) in accessions of *T. urartu* from Transcaucasia showed a striking resemblance to similar bands in the patterns of *T. dicoccoides* and *T. aestivum* (Fig. 2D, E, F, G). The slow region of the polyploid patterns was characterized by the absence of strong, discrete bands and fit best with species of *Aegilops*.

Considered together, these data provide support for *T. urartu* as a genome donor to tetraploids of the emmer type and to *T. aestivum*. Patterns of some accessions of *T. boeoticum* were also moderately similar to those of the emmer-*aestivum* polyploids, but those of *T. urartu* seemed to fit best. Our data also tend to support a contribution by some species of *Aegilops* to the emmer tetraploids. If proteins of *T. urartu* dominated in the intermediate region and those of *Ae. speltoides* dominated in the fast and slow regions, then the combined patterns of some accessions of *Ae. speltoides* and *T. urartu* would fit reasonably well with that of the tetraploid.

Johnson (1975) has proposed that the *A* and *B* genomes of emmer-*aestivum* type wheats were contributed by *T. boeoticum* and *T. urartu*, respectively. But Konarev *et al.* (1976) and Vittozzi and Silano (1976) have provided strong evidence that *Ae. longissima* donated the *B* genome to such wheats. Our results support the likelihood that *T. urartu* contributed one genome to these polyploids, but suggest some conflicts in assigning *T. boeoticum* as the other contributor. Moreover, all accessions of *Ae. longissima* differed substantially from the emmer-*aestivum* wheats throughout the intermediate and slow regions of their patterns (Figs. 3C, D; 4B, D; 5A, D). Therefore, if *Ae. longissima* did indeed contribute the *B* genome, the contributing form either was very different from ours or underwent a substantial loss of components upon (or after) polyploid formation.

Both a polyphyletic origin of polyploid wheats (Kimber and Athwal 1972; Johnson 1972; Vittozzi and Silano 1976; Zohary and Feldman 1962) and/or introgression between the same or different levels of ploidy (Vardi 1973) could explain some conflicts that have arisen. Diversity among diploid ancestors could have led to the formation of several amphiploids having the same genome complement, *AABB*. For example, two accessions of *T. urartu* could have hybridized with two species of *Aegilops*, e.g., *Ae. speltoides* and *Ae. longissima*, to form different amphiploids. If the resulting amphiploids intercrossed,

a recombination of genes might occur at the tetraploid level. Moreover, Vardi (1973) observed that hybridization and introgression between different levels of ploidy occurred not only in the laboratory but in nature. As a result of introgression from tetraploid to diploid, chromosome segments were transmitted to an entirely different diploid. Such a mechanism could account for genetic diversity among diploids as well as our present inability to associate a specific diploid with a definite genome.

Another possibility that deserves consideration is that some genes may be either unexpressed or lost during amphiploid formation. Some evidence suggests that protein components expressed in the amphiploid represent the sum of parental components (Johnson and Hall 1965) but we think this question should be investigated further.

Patterns of *T. araraticum* and *T. zhukovskyi* were highly similar to one another but distinctly different from those of *T. dicoccoides* and *T. aestivum*; these latter were also highly similar to one another. The derivation of the hexaploid species of each pair from the tetraploid is clearly evident in our electrophoretic patterns. The assignment of *T. boeoticum* and *Ae. speltoides* as donors of the *A* and *B* genomes to tetraploids of the *timopheevi* type (which include *T. araraticum*), as proposed by most recent workers (Johnson 1975; Konarev et al. 1976, Vitozzi and Silano 1976), would fit reasonably well with our results. We cannot rule out *T. urartu*, however, as a possible donor to wheats of the *timopheevi-zhukovskyi* types.

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

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