Variations in Soluble Endosperm Proteins of Corn (*Zea mays* L.) Inbreds as Detected by Disc Gel Electrophoresis[1]

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ABSTRACT

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The possibility that mutant and normal endosperms of corn (*Zea mays* L.) differ predictably in the electrophoretic banding patterns of their soluble endosperm proteins was examined by polyacrylamide gel electrophoresis. A band designation system was developed. One band was identified as sucrose synthase, and other bands were characterized according to special chemical or physical properties. Four banding patterns were noted among 15 normal inbreds. Each *opaque-2* pattern was different from that of the corresponding normal inbred, but no major single band or pattern change distinguished the *opague-2* inbreds as a group. The *floury-2* mutation caused a reduction in the amount of a major protein. Several examples of differential genetic control of relative protein levels are presented.

Corn (*Zea mays* L.) endosperm proteins can be conveniently divided into three major classes on the basis of solubility: salt-soluble, zeins or alcohol-soluble, and glutelins, defined either as alkali-soluble or saline and alcohol-insoluble proteins. The low lysine content of zein, the major storage protein, is responsible for the low nutritional value of corn, but several mutants, including *opaque-2*, *opaque-7*, and *floury-2*, have a greatly reduced proportion of zein in the endosperm. Increased amounts of the other proteins then cause an increase in lysine content (Misra et al 1972, Sodek and Wilson 1971). Yield and other agronomic characters are impaired in these mutants (Baenziger and Glover 1979), however, and an intense effort is being made to learn more about the developmental processes that occur in normal and mutant endosperms. The present study was designed to identify some of the salt-soluble proteins and to determine whether their amounts in zein-inhibited mutants differ from those in normal endosperms. Gel electrophoresis patterns may show that certain proteins are characteristic of mutants, and such proteins would be useful as markers in breeding programs. The original mutant lines of corn—*opaque-2* and *floury-2*—have soft floury endosperms. Some modified *opaque-2* endosperm types have vitreous regions similar to those in normal corn (Robutti et al 1974), but the basis of the changed appearance is not known.

Previous electrophoretic studies on endosperm proteins have generally compared albumins with globulins from a single line (Paulis and Wall 1969) or normal endosperm with one or more mutant endosperm types of isogenic backgrounds (Di Fonzo et al 1977, Paulis et al 1975). Many different inbreds and hybrids of corn exist, but variability among them with respect to types of protein has not been well examined. Recently Righetti et al (1977) reported that zein polypeptides vary greatly among genotypes, especially when examined by isoelectric focusing. Whole seed sodium dodecyl sulfate (SDS) extracts from two cultivars revealed differences after SDS-electrophoresis (Stegeman 1977). Corn germ contains a high proportion of salt-soluble proteins, and they differ somewhat from those in the endosperm (Paulis and Wall 1969). The salt-soluble proteins from three widely differing corn lines as well as from two strains of teosinte (*Zea mexicana* (Schrader) Kuntze) were separated by starch gel electrophoresis and found to be similar qualitatively but somewhat different quantitatively (Paulis and Wall 1977). The work I report here showed that appreciable differences among the salt-soluble proteins of corn inbreds can be detected after their separation by polyacrylamide gel electrophoresis (PAGE) and that the *opaque-2* and *floury-2* mutations cause various responses among the several types of salt-soluble proteins present. A preliminary report has been presented elsewhere (Wilson 1978).

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MATERIALS AND METHODS

The corn plants were grown in the field at Urbana, IL, and were hand-pollinated. Some *opaque-2* and *opaque-7* ears were pollinated with a mixture of *opaque-2* or *opaque-7* and normal pollen of the same inbred (mixed pollination). The ears were harvested about 50 days after pollination, unless otherwise specified, and were placed in plastic bags, chilled, and stored at −15° C. Most samples were at or near the stage of physiological maturity, as indicated by the beginning development of a black layer at the base of the kernel (Daynard and Duncan 1969). The

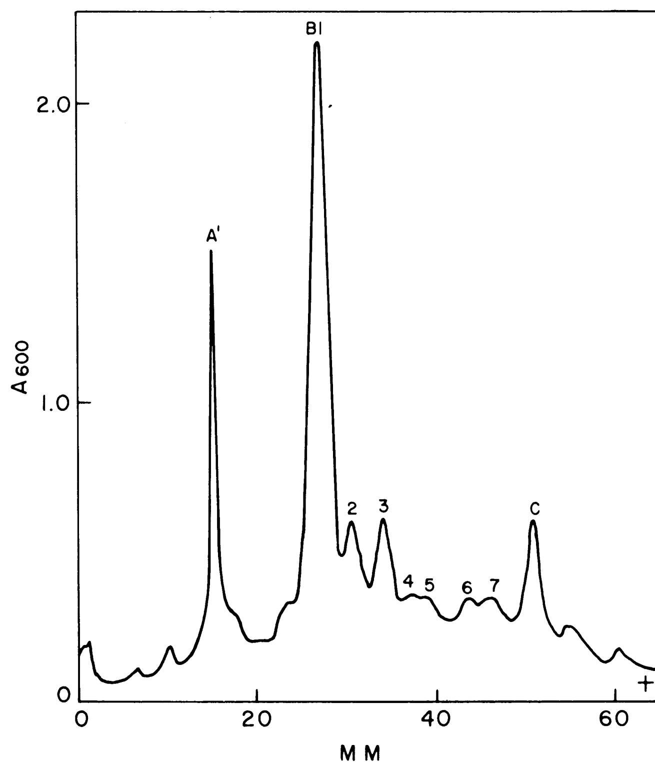
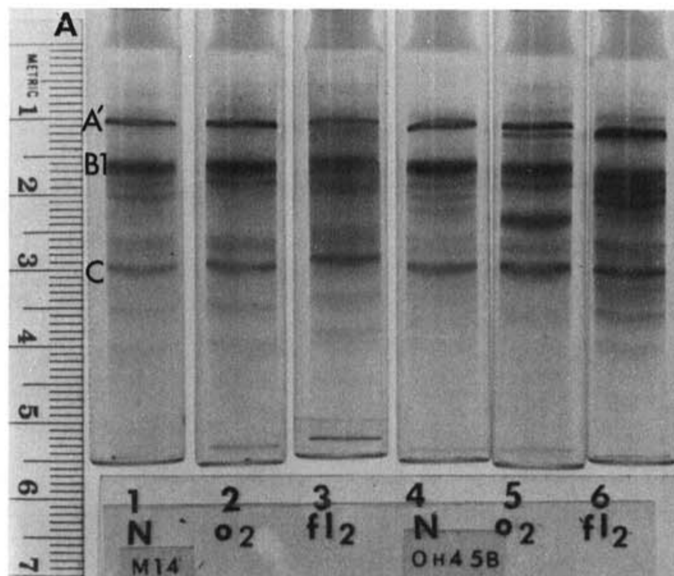
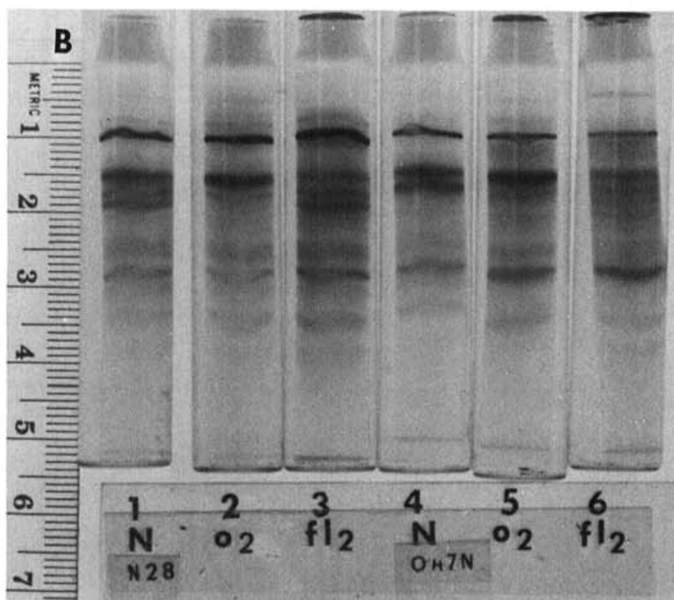


Fig. 1. Designation of protein bands after polyacrylamide gel electrophoresis of an endosperm extract from the cross M14 × Oh43, run on a 90-mm gel for 64 min. **A′** = sucrose synthase, the major band in the upper portion of the gel, **B1** = the major marker protein band for the middle or B region of the gel, **C** = the major marker band for the fast-moving proteins, **B2–7** = minor bands in the middle region.





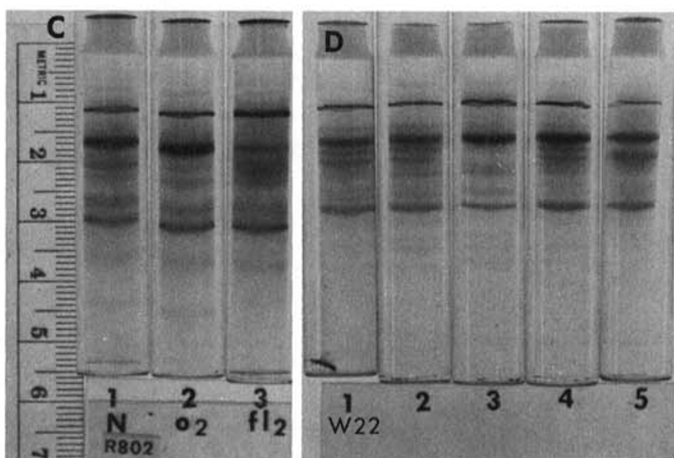


Fig. 2. Comparative polyacrylamide gel electrophoresis. **A–C,** Soluble proteins from normal, *opaque-2*, and *floury-2* endosperms of five inbreds harvested 50 days after pollination. **D,** Soluble proteins from the inbred W22: Gel **1,** homozygous normal; gel **2,** *opaque-2* pollinated by normal; gel **3,** homozygous *opaque-2*; gel **4,** *opaque-7* pollinated by normal; gel **5,** homozygous *opaque-7*. **A′** = sucrose synthase, **B1** = major band in middle region of gel, **C** = major band for fast-moving proteins.

endosperm dry weights of normal grains were between 65 and 80% of the fresh weight and were slightly lower for *opaque-2* endosperms.

For assay, the seeds were thawed and the endosperms separated from the seed coats and embryos. Usually three endosperms per sample were weighed, sliced with a razor blade, and soaked overnight at 3° C in three volumes (1.5–3 ml) of Tris-glycine sample buffer (0.005 *M* Tris and 0.0385 *M* glycine, pH 8.3) containing 10% sucrose. Overnight soaking improved homogenization and band resolution, especially for the more mature samples. The endosperms were homogenized for about 30 sec with the PT 10 ST generator of a Brinkmann Polytron. After equilibrating at 3° C for 1 hr, the homogenate was centrifuged at 6,000 *g* for 15 min. The supernatant solution could be immediately assayed or stored at −15° C for some months with little or no change in the electrophoretic patterns.

Approximate protein contents of supernatant solutions were determined by a semi-quantitative dye-binding test with amido black.[2] Comparisons were then made among gels loaded with about the same amount of protein (usually 60 μg); thus, differences in amounts of extractable protein among genotypes were not examined.

The PAGE method of Davis (1964) was used. The pH-6.7 large-pore (spacer) gel served as a final filter that prevented the top of the gel from clogging with material that could not enter the main gel. The main gel (pH 8.9 Tris-HCl buffer) contained 6.8–7.2% total acrylamide, with 2.6% bis-acrylamide cross-linking. The electrode buffer was twice as concentrated as the sample buffer and had no sucrose. Extracts were diluted with sample buffer containing a low concentration of bromphenol blue and were then layered on top of the gel under the electrode buffer. The apparatus allowed the gel tubes (5 mm i.d.) to be immersed in cold buffer surrounded by circulating ice water. Resolution was less sharp if the buffer was not kept cool. Electrophoresis was started at 2 mA per tube for 10 min, then continued at 4 mA per tube for about 35 min, by which time the dry front had usually reached 50–55 mm from the top of the main gel. The gels were stained and fixed overnight with 0.1% highly purified amido black (CI 20470, Acid Black 1) in 7% acetic acid, destained by transverse electrophoresis in 7% acetic acid, and stored in 7% acetic acid containing 1 mg/L amido black. Full details with scanning and photographic techniques are described elsewhere (Wilson 1979).

The major patterns reported for normal and *opaque-2* inbred endosperms were similar for samples from two or three ears harvested in each of three or four years. Most hybrids were tested at least two years, whereas the *floury-2* lines were tested only one year.

[2] Unpublished data.

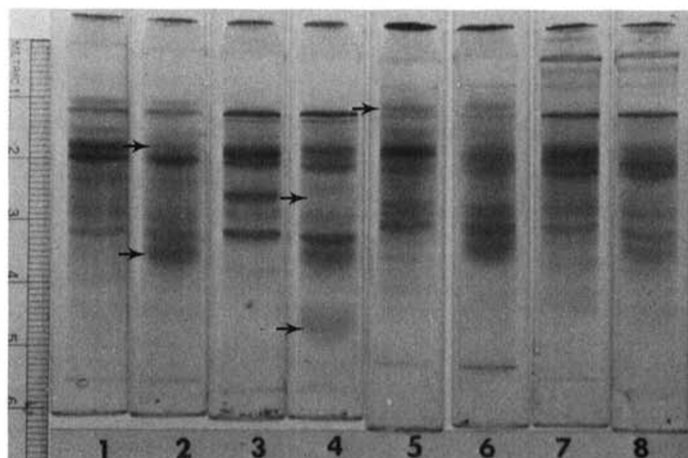


Fig. 3. Effect of dithiothreitol (DTT) on polyacrylamide gel electrophoresis of soluble proteins from four inbreds. The extracts for even-numbered gels contained 0.06 *M* DTT. Gels **1** and **2,** W64A; gels **3** and **4,** Oh45B-*o2*; gels **5** and **6,** M14 (mature); gels **7** and **8,** N28.

RESULTS AND DISCUSSION

Band Patterns of Normal Inbreds

A band designation system, illustrated by a scan of a long gel (Fig. 1), was developed so that different samples could be readily compared. Although some bands may have been due primarily to a single protein, even mature endosperm cells probably contained many more soluble proteins than indicated by the 20 or so bands seen under the best conditions. Thus, the band designations are those of positions on the gels, which were run under closely controlled conditions. The upper or A region of the gel contained one major band called A′. This band appeared to be distinctly different from all other bands; it was thin and membranelike and was easily affected by irregularities in gel composition. The next major band below A′ was B1—the first of seven bands in the middle or B region. Bands B2 and B3 were usually moderately intense but were as intense as B1 for some genotypes. Two pairs of minor bands (B4-B5 and B6-B7) were often indistinct. The fastest moving major band found for all genotypes was labeled C. The still-faster moving bands were minor, often broad and indistinct, and were not considered.

Under consistently controlled experimental conditions, reproducibility of banding patterns was good. The use of isolated endosperms rather than whole seeds eliminated the need for a preliminary extraction to remove lipids and also reduced the phytate content. The alkaline pH reduced both protease activity and interactions of protein with residual phytate (Craine and Fahrenholtz 1958). The present system, however, has some limitations. Only frozen endosperms were analyzed, and the endosperms represented only one stage of seed maturity (50 days) with a few exceptions. Extractability is probably higher for younger tissue; during the final stages of normal seed maturation, the albumins and globulins become more difficult to extract. Generally, seeds are extracted repeatedly and with larger volumes of buffer than the three volumes per weight I used. This, in turn, requires that the proteins be concentrated by precipitation, freeze-drying, ultrafiltration, etc. Problems may then arise in redissolving all of the protein from a precipitate or in preventing partial and differential precipitation during a concentration step without either denaturing some proteins or using a buffer that interferes with electrophoresis. I layered my samples directly onto the gel without further treatment; hence the albumins and globulins should not have been significantly altered. The homogenates obtained by use of the Polytron were quite fine and thus should have released close to the maximum amount of protein for the volume of buffer used. The amount of proteins in the normal endosperm extracts represented from 0.6 to 1.0% of the dry weight of the endosperms, whereas that in the *opaque-2* and *floury-2* extracts represented up to 1.5%. I did note losses in band sharpness and intensity, especially among the minor bands, when samples were homogenized in larger volumes of buffer or by glass-Teflon Potter-Elvehjem homogenizers.

Figure 2 shows the band patterns for six representative sets of inbreds with normal, *opaque-2*, and *floury-2* or *opaque-7* phenotypes. Except for the sample on gel 1 of Fig. 2D, all the normal samples were extracts of heterozygous endosperms produced by mixed pollinations on the same ears that furnished the homozygous *opaque-2* or *opaque-7* samples. No differences were noted between the PAGE patterns for heterozygous and homozygous normal endosperms. The A′ band at 10 mm was present for all endosperms harvested on day 50 or earlier. The B1 band (16 mm) probably contained the most protein in all normal and *opaque-2* samples because it was considerably broader, although often lighter, than the A′ band. The B2 and B3 band intensities, however, were characteristic for different normal inbreds. The most common pattern for normal endosperms (B1 ≥ B2 = B3) is illustrated by that for inbreds M14, Oh45B, and W22 in Fig. 2. This pattern was also found for Oh43, R803, B14A, and R801. A second pattern, B1 ≥ B3 > B2, was evident for R802 (Fig. 2C), B37, and WF9. The third pattern, B1 = B2 ≥ B3, was typical of Oh7N (Fig. 2B), W64A (Fig. 3, gel 1), Mo17, and B57. The last pattern, B1 ≥ B2 ≤ B3, was found for N28 only (Fig. 2B). The B4 band was more distinct for Oh45B than for the other samples, and the B5–B7 bands were often indistinct.

In comparison with the patterns for 50-day-old endosperms, those for younger endosperms often differed somewhat in relative band intensities and were higher in general background proteins (not shown). In the gels for fully mature endosperms, especially those air-dried for long-term storage, the A′ band was greatly reduced or missing (Fig. 3, gels 5 and 6), the B2 band was often reduced, and some bands were more distinct.

Mutant Band Patterns

The banding patterns for the *opaque-2* endosperms differed consistently from those of the corresponding normal endosperms in only one minor respect (Fig. 2). The B6-B7 pair of bands for the *opaque-2* sample became less distinct and, when scanned, often produced a single, rather broad peak. Otherwise, different inbreds responded differently to the *opaque-2* allele. M14-*o2* and R802-*o2* lost the B3 band. The most distinctive band change appeared in Oh45B-*o2*; bands B3 and B4 became indistinct, band B2 increased, and a new major band appeared in the B5 position. No conclusion could be reached as to whether this represented a new protein species or a great increase in the same B5 protein found in normal endosperms. Also, a new minor band (A″) appeared just under the A′ band, with the same thin, membranelike appearance as the A′ band. In the gel for N28-*o2*, the B3 band was lost and the B2 band greatly reduced; similarly, the B2 band for Oh7N-*o2* was greatly reduced. W22-*o2* appeared to have lost both the B2 and B3 bands but, in fact, may not have. The concentration of total soluble proteins was always greater in the *opaque-2* extracts; in compensating for this overall increase by diluting the sample, I may have diluted beyond detectability those proteins whose levels had remained low.

The banding patterns for the homozygous *floury-2* versions of the five sets of inbreds did show a major characteristic change from the patterns for normal endosperms (Fig. 2). Relative to all other bands, the B1 band decreased in intensity so that it was no longer as dark or broad as in both normal and *opaque-2* endosperm patterns. In addition, the general background staining increased throughout the portion of the gel where the bands were found.

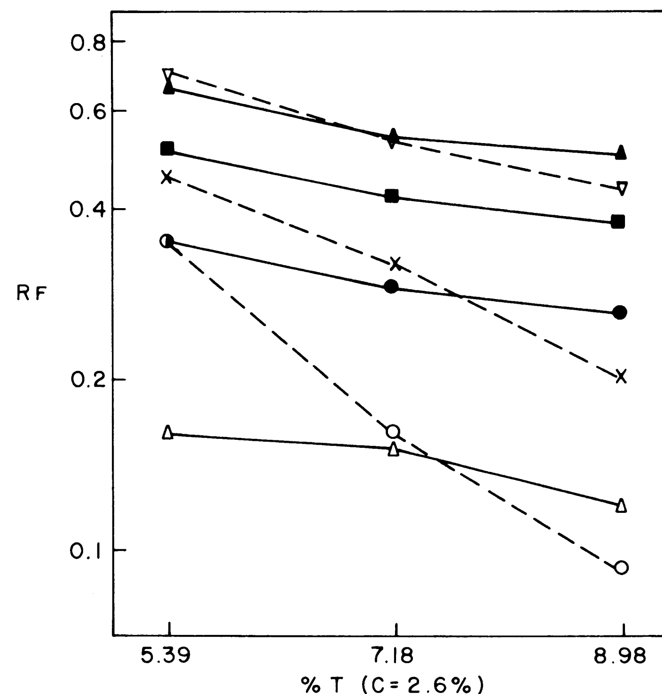


Fig. 4. Mobilities relative to the dye front (R_f) of endosperm proteins in gels of different polyacrylamide gel concentrations. ▽ and ▲ = C band proteins, ■ = B5 band, × = B2 band, ● = B1 band, O = A′ or sucrose synthase band, and △ = blue-green band from a mature endosperm. T = total acrylamide, C = % bis-acylamide cross-linking.

The *opaque-7* version of W22 (Fig. 2D, gel 5) differed from both the normal and the *opaque-2* versions. The intensity of the B2 band was decreased, but the B3 band remained.

Although several distinctive patterns were detected, a number of inbreds shared common patterns. A few patterns were unique (among those obtained so far), such as that for Oh45B-*o2*. Among the patterns for the normal genotypes, the one for N28 was unique but did resemble the patterns for Oh45B-*fl2* and N28-*fl2*. Several *opaque-2* inbred patterns resembled the one for M14-*o2*. The present separation technique may prove useful as part of a two-dimensional process of inbred identification.

Variable Protein Properties.

The banding patterns represented unknown proteins and protein mixtures, yet additional information about these proteins could be obtained. When the samples were made 0.06*M* in dithiothreitol (DTT) before being layered on the top of the gel (Fig. 3, even-numbered gels), the major B1 band was greatly reduced and a broad somewhat indistinct band appeared below the C band (arrows, gel 2). Also in the case of Oh45B-*o2*, the major B5 band disappeared and was replaced by a broad, fast-moving band (arrows, gel 4). The small amount of protein remaining at the B1 location probably was different from the one that had left that location. No other changes in band pattern were noted. One explanation is that the major proteins in the B1 and B5 bands are oligomers normally held together by disulfide bonds. Reduction of the disulfide bonds by DTT apparently released the smaller and thus faster-moving monomers.

During early stages of this work, the gels were stored in 7% acetic acid without added amido black. Sometimes the B1 and Oh45B-*o2* B5 bands faded over a period of days or weeks and could not be restained. This band disappearance was prevented by the addition of a small amount of dye to the storage solution. Apparently, the addition changed the equilibrium for dissociation of the dye-protein complex (Davis 1964, Wilson 1979). The fact that only these two bands faded also suggests that they are similar in some properties.

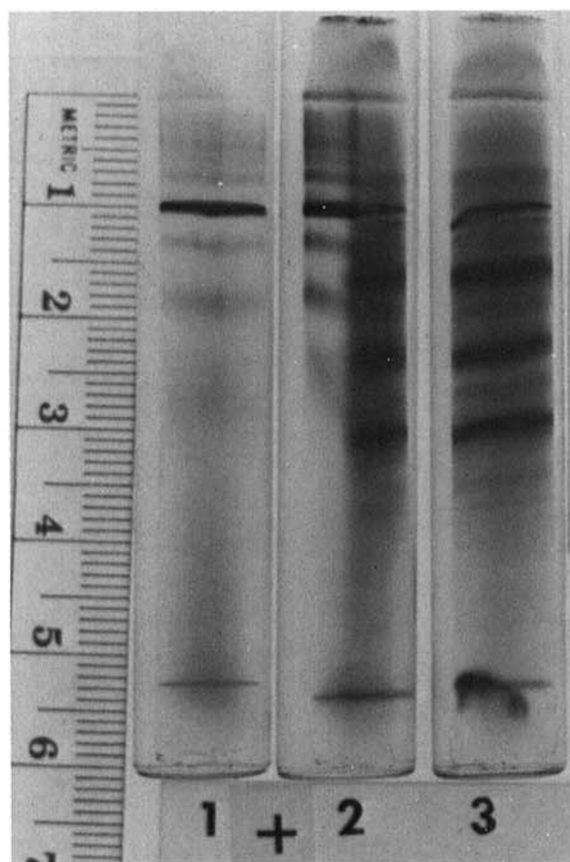


Fig. 5. Comparative polyacrylamide gel electrophoresis of sucrose synthase and a crude endosperm extract from the inbred Oh45B-*o2*. Gel **1**, Purified sucrose synthase; gel **2**, split gel with sucrose synthase on the left, endosperm extract on the right; gel **3**, crude endosperm extract.

The M14 extract used in Fig. 3, gels 5 and 6, was prepared from a fully mature ear, and the A′ band was extremely weak. Just above the A′ band was another band (arrow). It stained blue-green and was not seen in any 50-day samples. The possible significance of the color is discussed elsewhere (Wilson 1979).

Hedrick and Smith (1968) reported a procedure for electrophoretically characterizing proteins according to molecular weight and charge. I examined corn endosperm extracts by a simplified version of their method, using only three concentrations of gel (all components of the gel were unchanged except for total acrylamide concentration). These results, taken from several banding patterns involving several genotypes, are summarized in Fig. 4. The four bands whose R_f values are connected by solid lines (which are more-or-less parallel) probably represent proteins of similar size but with different numbers of charges. These are the B1 and B5 bands, one C band, and the blue-green band for mature endosperm. Nonparallel lines are formed by proteins that differ in size, and the steepness of the slopes increases with protein size; thus, the dashed lines represent the larger proteins. Apparently the A′ protein was the largest major protein. It was identified as sucrose synthase and has been shown to have a molecular weight of 365,000 (Su and Preiss 1978). The lines connecting R_f values for the A″ and the A′ bands of Oh45B-*o2* were parallel (not shown). The concomitant disappearance of the A′ band and appearance of the blue-green band suggested that the staining properties of the A′ band had changed. However, Fig. 4 clearly shows that the A′ and the blue-green-staining proteins, which almost comigrated in the 7.18% gel, were not the same. The figure also shows that the major C band in 7.18% gels actually comprised at least two bands. The larger protein (dashed line) formed a more distinct band than did the other C band protein(s).

The B2 protein band moved only slightly faster than the B1 band in the 7.18% gel. Because the B1 band was broad, the B2 band sometimes appeared only as a shoulder on the side of the B1 band when the gel was scanned, and was indistinct in photographs when the separation between these two bands was small. The pore size of polyacrylamide gels is affected by catalyst type, age, and concentration, temperature during gelation, reagent purity, weighing and pipetting precision, etc., and thus may vary slightly among batches of reagent solutions, runs, and laboratories. If the B1-B2 bands overlapped in a series of PAGE runs, a slight reduction in the amount of acrylamide improved the separation. The Davis (1964) disc gel electrophoresis system is widely used and should give similar results wherever it is used. Dalby et al (1972) reported gel banding patterns for extracts from 22-day-old W64A normal and *opaque-2* endosperms. The gel for the normal endosperm clearly showed the A′, B1, B2, and C bands, and the gel for the *opaque-2* endosperm showed the loss of the B2 band. The patterns were similar to those I obtained using 50-day-old endosperms from W64A normal (Fig. 3, gel 1) and *opaque-2* (not shown), which are like those from Oh7N normal and *opaque-2* endosperms (Fig. 2B).

Chourey and Nelson (1976) reported that the shrunken (*sh*) mutation is specific for a deficiency in sucrose synthase.[3] A major protein band was missing from both starch gel (Schwartz 1960) and Davis-type polyacrylamide gel (Chourey and Nelson 1976) patterns for *sh* extracts. This band appeared to correspond with the band I have designated as A′. The correspondence was confirmed when I analyzed extracts, provided by Dr. Chourey, from both normal and *sh* endosperms (data not shown). I also electrophoretically examined a sample of purified sucrose synthase (provided by B. Burr, Brookhaven National Laboratory) after dissolving it in sample buffer containing 0.01 *M* KCl and 0.06 *M* DTT. The sucrose synthase comigrated with the A′ band of an Oh45B-*o2* extract (Fig. 5). Sucrose synthase is normally present as a tetramer (Su and Preiss 1978), the species apparently responsible for the major band in gel 1 of Fig. 5. When dissolved in sample buffer containing 3 *M*

[3]Sucrose synthase was E.C. 2.4.1.13, not E.C. 2.4.1.14 as given in Chourey and Nelson (1976).

urea, the purified enzyme gave no single major band but rather a series of eight or more bands (data not shown); presumably, the fastest-moving one was due to the monomer weakly seen in Fig. 5.

The *sh* mutant gels had a "forward band," which moved slightly faster than sucrose synthase and which may have a different sucrose synthase activity (Chourey and Nelson 1976). The gel for the *sh* extract was compared with that for an Oh45B-*o2* extract, the latter containing the A″ band (slightly faster than the A′ band, as evident in Fig. 5, gels 2 and 3). The *sh* forward band migrated between the A′ and the A″ bands, as indicated by visual observations, but a satisfactory scan or photograph could not be obtained.

A protein (band X) that was not fixed during the standard amido black staining procedure was found in the *floury-2* version of inbred R802 (Wilson 1979). It is not visible in gel 3 of Fig. 2C. This unusual major protein was detected when most, but not all, of the excess dye was removed by rinsing rather than by electrophoresis (Fig. 6), but it disappeared in another day or so. Band X protein was fixed by trichloroacetic acid and could be stained by Coomassie blue R but not by amido black. The Coomassie blue R-protein complex was bluish, unlike the other stained proteins on the same gel (Wilson 1979), but the special properties of this protein that caused the metachromatic effect are unknown. Six other inbreds available in the *floury-2* version were tested for band X, but it was absent. The inbred R802 was developed from a cross to the Illinois High Oil strain. Protein X was found in some but not all tested samples of mature seed of the Illinois High Oil strain and also in some R801 normal and *floury-2* lines (R801 was also derived from an Illinois High Oil cross). No genetic study has yet been made, but the results suggest that the protein X allele is not linked to the *floury-2* mutation in which it was first noted and that it may be segregating within lines derived from Illinois High Oil.

Although the trichloroacetic acid-Coomassie blue R fixation and stain method was needed to fix band X protein at least semipermanently, it was less convenient to use routinely than was 7% acetic acid with amido black. Coomassie blue R solvent systems containing alcohol did not fix band X protein and fixed bands B1 and B5 rather poorly (Wilson 1979).

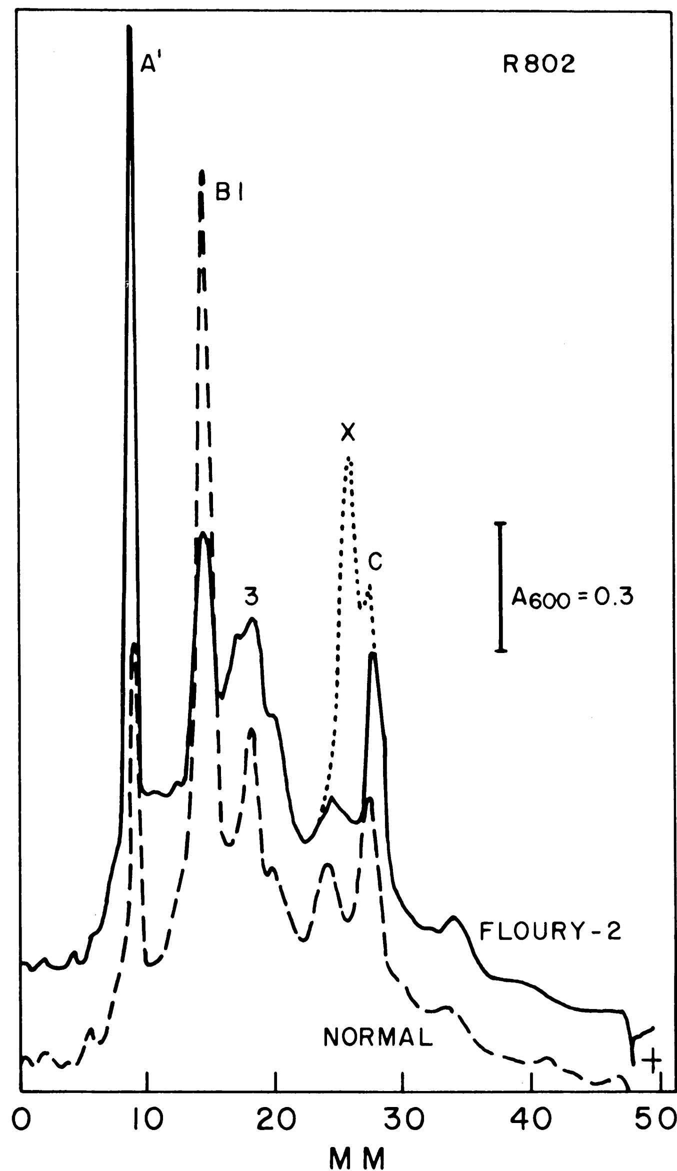


Fig. 6. Polyacrylamide gel electrophoresis of a poorly fixed protein, band **X** in R802. Scans of patterns of normal (----) and *floury-2* (——) soluble proteins were made after gels were destained electrophoretically. ···· = portion of the scan of a gel with *floury-2* protein that was destained by rinsing with 7% acetic acid until most but not all of the excess amido black was removed. Baselines for the scans were different; the rinsed gel scan was adjusted to coincide with the corresponding electrophoretically destained scan. **A′** = sucrose synthase, **B1** = major band in middle region of gel, **C** = major band for fast-moving proteins, **B3** = a band in middle region of gel.

Hybrid Band Patterns.

When two inbreds were crossed, the banding patterns for the resulting hybrid endosperms often could be recognized as intermediate between those for the two parents. I examined the relative levels of the B1, B2, and B3 bands for normal inbreds (Fig. 7). The patterns for inbreds M14 (scan 1) and B14A (not shown) were similar, showing about equal levels of B2 and B3. When M14 was pollinated by B14A, the hybrid pattern was similar to the patterns for both parents (scan 2). Pollination of M14 by Oh7N, which had a strong B2 band (scan 5), resulted in a hybrid with a B2 band that was appreciably more intense than the B3 band (scan 3), whereas pollination by N28, which had a strong B3 band (scan 6), resulted in a hybrid with a B3 band more intense than the B2 band (scan 4). The peak heights agreed with the expected gene dosages in the triploid endosperm. The traits for a strong B2 band in Oh7N and for strong B2 and B3 bands in N28 were less evident in their progeny, which had M14 or a similar inbred for the pollen parent, but variations in band height often made distinguishing the hybrids from inbreds difficult. On the average, however, a preliminary statement that complete maternal dominance existed (Wilson 1978) was not confirmed.

The *opaque-2* version of the inbred Oh45B responded differently to different foreign pollens. Its PAGE pattern was characterized by

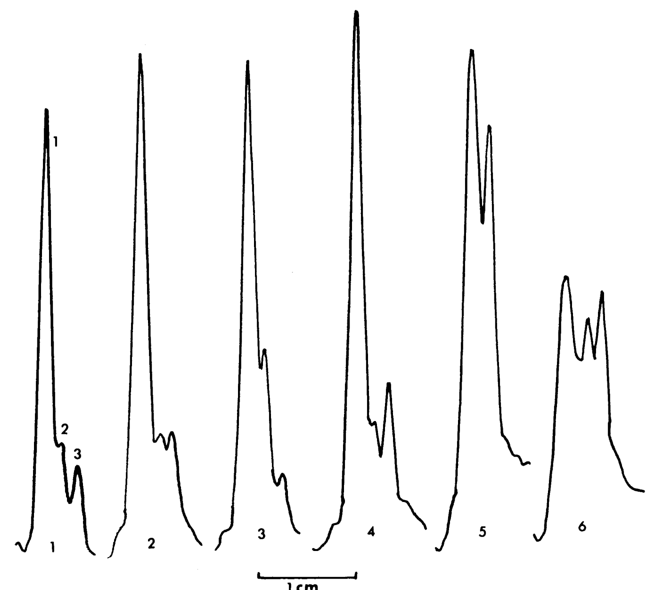


Fig. 7. Genetic variability in the intensities of bands B2 and B3. Portions of the scans of gels including B1, B2, and B3 bands are shown for three inbreds and three hybrids. Scan **1,** M14; scan **2,** M14 × B14A; scan **3,** M14 × Oh7N; scan **4,** M14 × N28; scan **5,** Oh7N; and scan **6,** N28.

the presence of a very large B5 band, a strong C band, and a small A″ band (Fig. 8, scan 1). The pattern for the hybrid Oh45B-*o2* × Oh7N-*o2* (scan 2) showed those three characteristic bands, but they were at lower levels than expected from the double dose of Oh45B-*o2* genes. For the hybrid Oh45B-*o2* × M14-*o2* (scan 3), however, the three characteristics of the maternal parent were completely absent. Several hybrids with other *opaque-2* pollen parents produced patterns much more like those of the pollen parent than of Oh45B-*o2*. The characteristic elements of the Oh45B-*o2* pattern did not show up in the pattern for any hybrid with this inbred as the pollen parent. The pattern for hybrid Oh45B-*o2* × W64A-*o2* showed a low level of the B5 band (Fig. 9, scan 2), whereas the reciprocal cross was almost identical with the inbred W64A-*o2* (scans 3 and 4). The A″ band was not evident in the pattern for either hybrid, but the B6-B7 doublet enlarged progressively as the W64A-*o2* dosage increased. The four samples, of three endosperms each, that provided the extracts for Fig. 9 were taken from only two ears, which had been pollinated with a mixture of Oh45B-*o2* and W64A-*o2* pollen. The kernels for samples 1 and 2 were selected on the basis of size, the selfed Oh45B-*o2* endosperms averaging 165 mg (fresh weight) and the hybrid endosperms, 300 mg. The endosperms on the W64A-*o2* ear were selected on the basis of both size (selfed, 210 mg; hybrid, 160 mg) and endosperm color. These scans were compared with those for endosperms from ears pollinated with only one pollen parent, and the comparisons confirmed that the genotype assignments for the kernels from the mixed ear were correct. The weight differences between the inbred and hybrid endosperms were also found when they were taken from different ears.

The limited observations on hybrids of normal genotypes suggested that at least some of the proteins were present in proportion to the dosage of parental genes (Fig. 7). The results involving Oh45B-*o2* as the maternal parent, however, showed a strong effect of the pollen parent on the resulting gel patterns. Davies suggested that selective repression or activation of parental loci could occur in pea hybrids (Davies 1973). The synthesis of the specific proteins in the Oh45B-*o2* endosperm was partially or completely repressed by foreign pollen. Further tests are underway to determine the reason for the variable expression of the paternal phenotype in the hybrid, possibly a nonallelic gene interaction.

Major changes in protein and zein contents of corn endosperm are controlled in very different ways. The recessive *opaque-2*

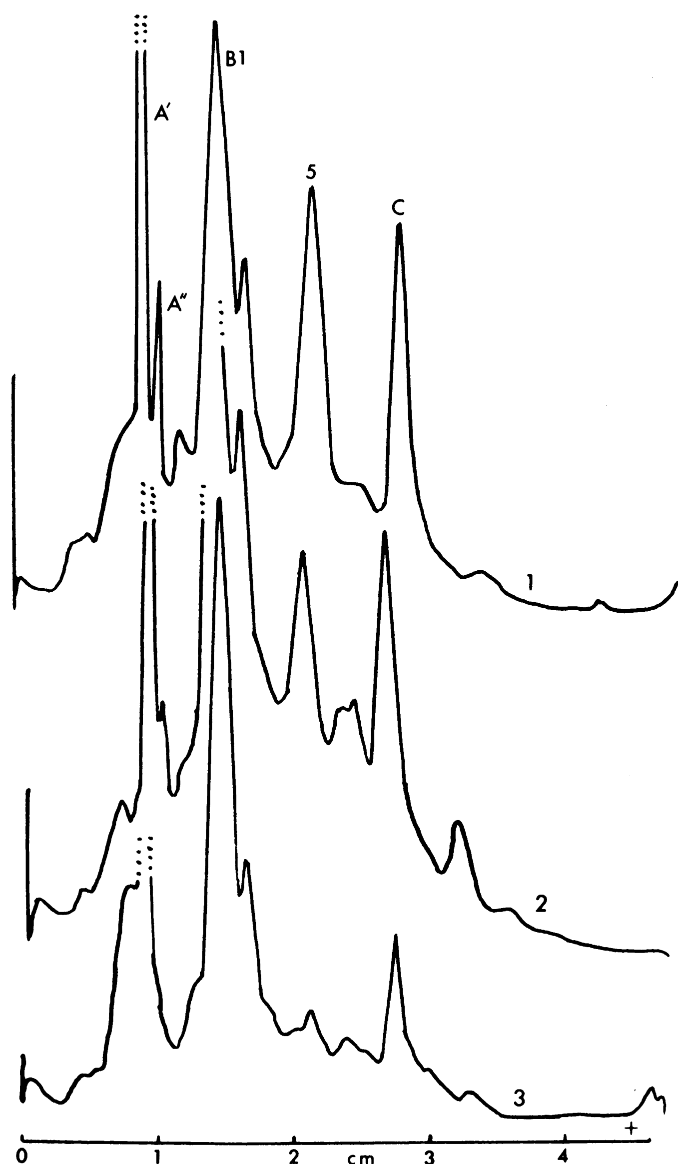


Fig. 8. The effect of two *opaque-2* pollen parents on the banding pattern of Oh45B-*opaque-2* endosperm proteins. Scan **1**, Oh45B-*o2* selfed; scan **2**, Oh45B-*o2* × Oh7N-*o2*; scan **3**, Oh45B-*o2* × M14-*o2*. **A′** = sucrose synthase, **A″** = minor band in top region, **B1** = major band in middle region, **B5** = a band in middle region, **C** = major band for fast-moving proteins.

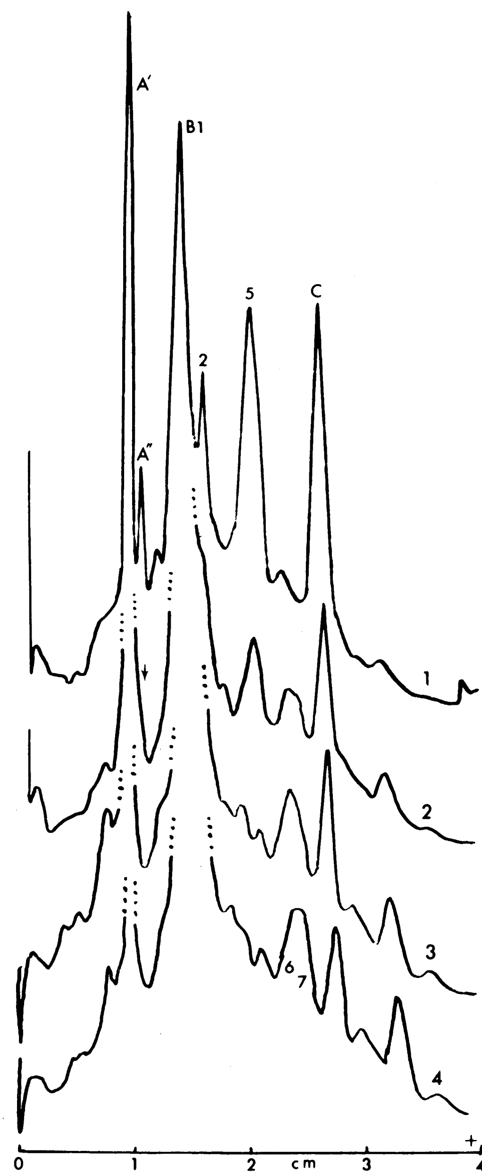


Fig. 9. Polyacrylamide gel electrophoresis banding patterns for the inbreds Oh45B-*o2* and W64A-*o2* and their reciprocal crosses. Scan **1**, Oh45B-*o2*; scan **2**, Oh45B-*o2* × W64A-*o2*; scan **3**, W64A-*o2* × Oh45B-*o2*; scan **4**, W64A-*o2*. **A′** = sucrose synthase, **A″** = minor band in top region, **B1** = major band in middle region, **B2** and **5–7** = bands in middle region, **C** = major band for fast-moving proteins.

mutation greatly reduces zein content and protein body size (Wolf et al 1969) and increases lysine content (Misra et al 1972, Paez et al 1970, Sodek and Wilson 1971). The *floury-2* mutation reduces zein (Jones 1978) and increases lysine (Paez et al 1970) in proportion to the number of *fl2* alleles and also disrupts protein body formation (Christianson et al 1974). The protein contents of Illinois Low Protein and Illinois High Protein strains, with about 5 and over 17% protein, respectively (and corresponding low and very high zein contents), were almost unaffected by pollen from lines with different protein contents (Leng et al 1951, Wilson.[4])

Related Considerations

The only protein band that was identified with an enzyme band was A′, and the enzyme was sucrose synthase (Chourey and Nelson 1976). The results of genetic studies suggest that no other protein comigrates with sucrose synthase on the gel and that when the enzyme activity is missing, the band is missing also. This enzyme makes up about 2.8% of the soluble protein of corn endosperm, as estimated from purification experiments (Su and Preiss 1978). Such an estimate could not be readily made from PAGE patterns because the A′ band is thin and very intense; nevertheless, the patterns did show that A′ was a major protein. In contrast, an estimate based on purification experiments is that ribonuclease I (RNase I) in an unfractionated extract of corn endosperm would not be detectable by staining with amido black after gel electrophoresis (Wilson 1967). These estimates were based on the observations that sucrose synthase was considered pure after a 40-fold purification and RNase I, after a 4,000-fold purification. Some enzyme activities vary greatly among lines. For example, normal inbreds have a six-fold range of RNase activity and the *opaque-2* mutation extends the range to over 20-fold (Wilson 1978). Changes of this magnitude could change the protein content of certain enzymes (though not of RNase) enough that they would cross the detectability threshhold.

Studies on corn albumins and globulins usually show their highest amounts three to four weeks after pollination and somewhat reduced amounts in mature endosperms (Moureaux and Landry 1972, Sodek and Wilson 1970). Whether the proteins are broken down before maturity or are just rendered insoluble in the dry, mature seed has not been determined. If any of the proteins of the mature glutelin fraction are, in fact, some of the same proteins that had made up the globulin and albumin fractions earlier, those proteins might be identified by comparisons of banding patterns. However, extraction and PAGE systems different from those I used might be necessary. In preliminary experiments, I extracted proteins with water and with buffers of low salt contents, and then electrophoretically separated the extracts. I found no clear-cut differences between the albumin (water-soluble) and globulin (salt-soluble) banding patterns, although the relative intensities of certain bands differed. Because of extensive overlap, these two fractions could not be distinguished. "Globulins" could be precipitated from a salt extract by dialysis against water, but then redissolving all of the precipitated protein in a buffer that was compatible with the electrophoresis system became difficult. The albumins and globulins, together, have a higher lysine content than do corn endosperms as a whole (Sodek and Wilson 1971). Thus, a corn variety with above-average amounts of these proteins has an improved nutritional value. The occurrence in inbreds, such as Oh7N, W64A, N28, and Oh45B-*o2*, of high levels of certain soluble proteins (Figs. 2 and 3) suggests that these inbreds should be examined to determine whether these differences are sufficient to give an overall increase in the total amount of easily soluble protein.

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[4]Unpublished data.

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