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Protein Electrophoretic Profiles and the Origin of the B Genome of Wheat

(*Triticum*/*Aegilops*/amphiploids)

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ABSTRACT **Protein electrophoretic profiles cast doubt upon the prevalent theory that the B genome of the polyploid wheats was derived from a species of *Aegilops*. They suggest, instead, that the wild tetraploid wheats comprise a complex, whose components were derived from various combinations of diploid *Triticum* types, which evidently include the B-genome type.**

The A genome of cultivated wheats, including the emmer tetraploids (AABB) and the *aestivum* hexaploids (AABBDD), is related to that of the diploid wheats (AA) (1) comprising the wild Near Eastern *Triticum boeoticum* Boiss. and its derivative *T. monococcum* L., which was domesticated during Neolithic times. The D genome was contributed by *Aegilops squarrosa* L. (DD) (2, 3), a common invader of wheat fields from the Transcaucasus to Afghanistan. These genome affinities were deduced largely from the amount and kind of meiotic chromosome pairing observed in F_1 hybrids between the cultivars and their presumed diploid progenitors. While the origin of D is unequivocal, the homology between A of the polyploids and A of the diploids is incomplete (4).

The source of B is controversial. Camara (5) assumed that the tetraploids (AABB) may have arisen by autoploidy, B as well as A being derived from the diploid wheats. Various geneticists speculated that B may have been contributed by a species of *Agropyron*. However, chromosome pairing has failed to demonstrate substantial homology between this genome and that of any suspected diploid donor (6). Hence, recent theories regarding the origin of B rest largely on morphological evidence.

From comparisons of spikelet morphology, Sarkar and Stebbins (7) concluded that the tetraploid wheats originated by amphiploidy involving the diploid wheats and *Aegilops speltoides* Tausch or a species very similar to it. However, Sears (8) noted that the amphiploid *Ae. bicornis–T. monococcum* resembled the tetraploid *T. dicoccum* Schübl. more closely than did the amphiploid *Ae. speltoides–T. monococcum*.

The hypothesis that *Ae. speltoides* was the B donor gained wide acceptance despite some evidence to the contrary. Riley, Unrau, and Chapman (9) concluded that the satellited chromosomes characterizing the B genome indicated its origin specifically from *Ae. speltoides*. They also suggested that a close relationship can be inferred between the chromosomes of *Ae. speltoides* and those of the B genome, considering the observation that the *speltoides* genome suppresses the restricting effect of wheat chromosome 5B on intergenomic pairing. Sears (10) observed, however, that in the absence of the normal suppressing effect of the *speltoides* genome on 5B activity (11), *Ae. speltoides* failed to show appreciably more chromosome pairing with *T. aestivum* than did diploid species not closely related to A, B, or D. Hence, if *Ae. speltoides* was the donor of the *Triticum* B genome, the homologies of B, but not those of A and D, presumably, must have been altered by chromosome structural rearrangements following the origin of the primary amphiploid.

Nuclear DNA content (12) and the acid phosphatase (EC 3.1.3.2) electrophoretic pattern (13) based on limited sampling of the species were asserted to point to *Ae. speltoides* rather than to *Ae. bicornis* (Forsk.) Jaub. et Spach. as the donor of B.

A new approach to the problem of the B donor was provided by the observation (14, 15) that the parents of a synthetic amphiploid could be identified from the band pattern produced by electrophoresis of a seed-protein extract. This method applied to natural polyploids has confirmed the postulated parental species of the following *Triticinae* amphiploids, where cytogenetic methods provide reasonably unambiguous evidence of genome identity: *Ae. cylindrica* Host. (16), *Ae. triuncialis* L. and *Ae. triaristata* Willd. 6x (17), *Ae. juvenalis* (Thell.) Eig and *Ae. ventricosa* Tausch (18), *T. zhukovskyi* Men. et Er. (19), and *T. aestivum* L. em. Thell. (Johnson, B. L., manuscript submitted for publication). In the case of *Ae. crassa* Boiss. (conventionally $DDM^{cr}M^{cr}$), the protein pattern (18) confirmed cytogenetic evidence that the tetraploid was in fact genomically heterogeneous, and showed that the parents of one of the component profile types were *Ae. squarrosa* (DD) and *Ae. speltoides* (SS).

Precise identification of the parental types within variable populations depends on the principle that the protein-band pattern of an amphiploid qualitatively represents the summation of the parental patterns, and that the amphiploid pattern can be simulated by electrophoresis of a mixture of protein extracts from the parents. Although, presumably, not immutable in the course of evolution, the protein pattern provides another criterion of genome relationship possibly commensurate with chromosome-pairing affinity.

Preliminary assessments of genome homologies in wheat by means of the seed-protein electrophoretic pattern cast doubt on both *Ae. speltoides* and *Ae. bicornis* as donors of the B genome (15, 20). Evidence from subsequent studies of *Triticum* and *Aegilops* presented in this report indicate that the donor of the *Triticum* B genome was not a species of *Aegilops*, but was more probably a diploid wheat whose precise identification may require a detailed study of the wild diploid and tetraploid populations.

MATERIALS AND METHODS

Conclusions drawn in this report are based on protein electrophoretic profiles of all commonly recognized species of *Triticum* and *Aegilops*. The number of accessions examined is given in Table 1 only for the tetraploid wheats and for purported A- and B-genome donors and other diploids of close affinity.

Protein extracts of ground seeds of these accessions made with 70% ethanol (21) were freeze-dried for prolonged storage. The crude extracts were electrophoresed at pH 4.3 with β-alanine–acetic acid buffer on 15% acrylamide gels (22). A 0.5-mg sample of the dry protein powder was applied to each column and electrophoresed for 2 hr 15 min at 4 mA per column. The gels were fixed and stained with 0.5% amido black in 7.5% acetic acid and then photographed.

Minor discrepancies in migration velocity of homologous bands from gel to gel were corrected by adjusting all photographs to comparable lengths with reference to the pattern of *T. dicoccum* accession 497 used as a standard. To provide for identification of homologues, this method (21) required the electrophoresis of a 1:1 mixture of protein from the standard and the accession whose pattern needed adjustment. Band homologies between species were further verified by electrophoresis of mixtures of their protein extracts.

The protein profile of *Triticum* and *Aegilops* species obtained by the method described comprises two somewhat distinct series of bands. Less resolution is provided in the slower gliadin series (21, 23), between the origin and −4.0 cm; therefore, conclusions regarding band homologies are based largely on the faster moving albumin series comprising the rest of the pattern. The accessions included in Figs. 1–36 were selected to cover the range of profile variability observed among the tetraploid and diploid wheats, and the variability within individual diploid species of *Aegilops*.

TABLE 1. *Species of Triticum and Aegilops studied electrophoretically*

Wild species		Cultivars	
Triticum			
Emmer tetraploids (AABB)			
T. dicoccoides	(38)*	*T. dicoccum*	(58)
		T. turgidum	(7)
		T. durum	(30)
		T. turanicum	(11)
		T. polonicum	(11)
		T. carthlicum	(5)
		T. aethiopicum	(3)
Timopheevi tetraploids (AAGG)			
T. araraticum	(8)	*T. timopheevi*	(13)
		T. georgicum	(2)
Diploids (AA)			
T. boeoticum	(44)	*T. monococcum*	(36)
Aegilops			
Diploids of sections *Sitopsis* and *Amblyopyrum*			
Ae. speltoides	(71)		
Ae. bicornis	(17)		
Ae. longissima	(17)		
Ae. sharonensis	(8)		
Ae. mutica	(4)		

* The numbers in parentheses refer to the number of accessions examined.

RESULTS AND DISCUSSION

The tetraploid wheat protein profile

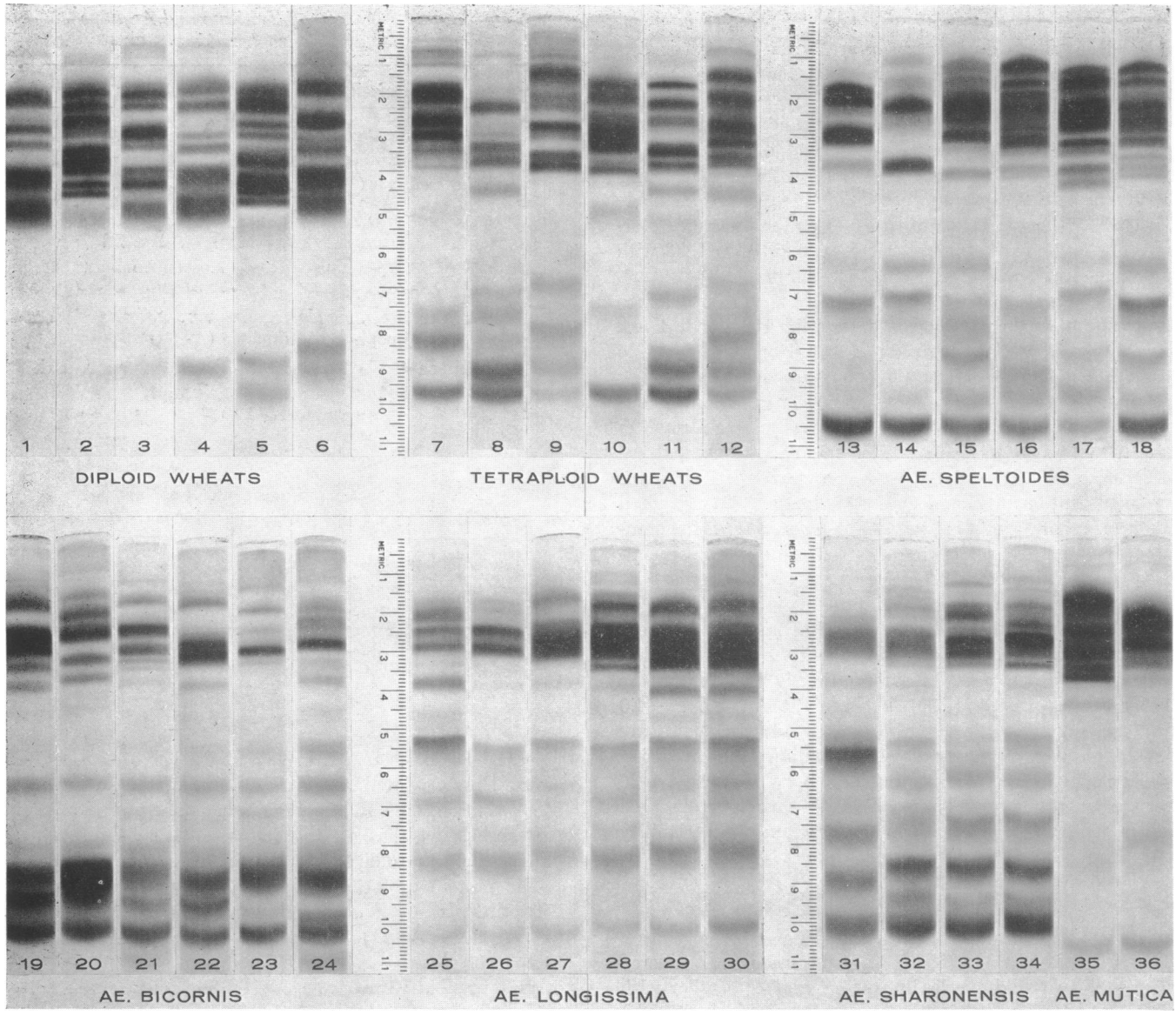
The tetraploid wheats (Figs. 7–12) are commonly considered to include two wild species (Table 1) which, together with their cultivated derivatives, comprise the emmer (AABB) and *timopheevi* (AAGG) groups. The protein pattern (Fig. 7) is notably uniform among species of the latter group. In the former, the various cultivars present essentially a single pattern (Fig. 12), whereas marked variability occurs in the wild species *T. dicoccoides* Körn. Schweinf. (Figs. 8–11). Despite this variability the tetraploid wheats are characterized by an albumin pattern of 3–7 distinct bands between −4.5 and −9.7 cm on the standardized scale. The fastest band is invariably at −9.7 cm.

Diploid *Aegilops* homologies of the tetraploid wheats

Aegilops speltoides (Figs. 13–18) has a remarkably uniform albumin pattern comprising seven or eight bands between −4.5 and −10.5 cm, six of which are homologous across all accessions. Thus, any random *speltoides* type functioning as the B donor would be expected to contribute at least these six albumin bands to the tetraploid wheats. However, a cursory comparison of the profiles shows that the mean number of *speltoides* albumin homologues missing in the tetraploid profile is about 5.6 out of 8. Similar comparisons involving the other species of the section *Sitopsis* including *Ae. bicornis* (Figs. 19–24), *Ae. longissima* Schweinf. et Musch. (Figs. 25–30), and *Ae. sharonensis* Eig (Figs. 31–34), as well as the closely related *Ae. mutica* Boiss. of the section *Amblyopyrum* (Figs. 35 and 36), show that none of these diploid patterns except that of *Ae. longissima* is substantially represented in the profile of the tetraploid wheats.

The protein profile of a synthetic amphiploid (Fig. 40) between *T. boeoticum* accession G560 (AA) (Fig. 37) and *Ae. bicornis* accession G365 (Fig. 38) bears little resemblance to that of either the tetraploid cultivars (Fig. 41) or any other tetraploid wheat (Figs. 7–11). This suggests that *Ae. bicornis* is not the donor of the *Triticum* B genome. This conclusion can be drawn equally well, however, from the profile of a 1:1 mixture of protein extracts (Fig. 39) from the parents of the amphiploid. Similar 1:1 protein mixtures involving various individual accessions of *Ae. speltoides* or other *Aegilops* species with individual accessions of *T. boeoticum* fail to simulate the tetraploid wheats.

Most strikingly, all diploid *Aegilops* species are characterized by a prominent albumin band that migrates ahead of the fastest band of the tetraploid wheats. This band falls at −10.5 cm in *Ae. speltoides* and at −10.2 in the other *Sitopsis* diploids. In *Ae. mutica* it falls at about −10.5, as it does in most of the remaining *Aegilops* diploids. Furthermore, in every instance among numerous accessions representing all known *Aegilops* polyploid species (17, 18), this characteristic band was present as expected. It was also present, as expected, in all *aestivum* (AABBDD) hexaploid wheats (21), which are known to carry the *Ae. squarrosa* (DD) genome, but it was absent, as expected, in *T. zhukovskyi* (AAAABB), which does not carry an *Aegilops* genome (19). Hence, the consistent absence of this band from all the tetraploid wheats seems to effectively exclude all known *Aegilops* species as the donor of the *Triticum* B genome.



FIGS. 1–36. Electrophoretic profiles of seed proteins of *Triticum* and *Aegilops* species. FIGS. 1–6. *T. boeoticum* accessions G640, G1016, G1074, G1195, G1546, and G1173, respectively. FIG. 7. *T. timopheevi* accession G383. FIGS. 8–11. *T. dicoccoides* accessions G372, G1453, G645, and G649, respectively. FIG. 12. *T. dicoccum* accession G563. FIGS. 13–18. *Ae. speltoides* accessions G762, G978, G1273, G1270, G943, and G861, respectively. FIGS. 19–24. *Ae. bicornis* accessions G549, G944, G1423, G1427, G1425, and G1420, respectively. FIGS. 25–30. *Ae. longissima* accessions G609, G945, G1306, G1305, G1304, and G1303, respectively. FIGS. 31–34. *Ae. sharonensis* accessions G946, G1315, G1322, and G1323, respectively. FIGS. 35 and 36. *Ae. mutica* accessions G983 and G985, respectively.

Variability of the tetraploid profile

Profile dissimilarities between emmer (AABB) tetraploids (Figs. 8, 9, 11, and 12) and *timopheevi* (AAGG) tetraploids (Fig. 7) suggest that these two groups differ with respect to their first as well as their second genome (24). Furthermore, tetraploids of uncertain affinity to either group (Fig. 10) have been collected, and aberrant profile types (Fig. 11) have been found in seemingly authentic emmer. These observations indicate that the *Triticum* tetraploids endemic to the Near East may represent a polyphyletic complex based on a number of more or less differentiated genomes. The extant tetraploid cultivars, virtually all of which can be characterized by a common protein profile (Fig. 12), seem to be derivatives of essentially only one (Fig. 9) of the several wild types (Figs. 7–11). Evidence from the protein pattern of the wild diploid wheats, which ostensibly contributed the A genome to the tetraploids, suggests that they also contributed B.

Diploid *Triticum* homologies of the tetraploid wheats

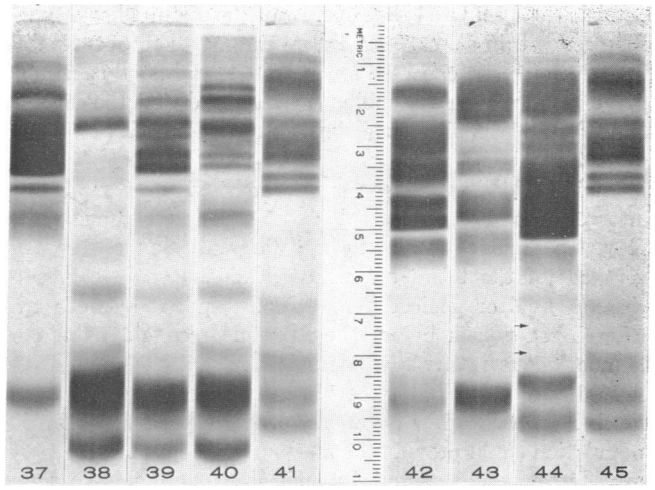
The diploid wheats may exhibit a short profile (Figs. 1 and 2) consisting mostly of gliadin bands, often with only two distinct albumin bands at −4.2 and −5.0 cm, or a long profile (Figs. 3–6) including one or two additional prominent albumin bands, one of which commonly occurs at −9.0 cm. However, many accessions show a shadow pattern of faint albumin bands usually not visible in published reproductions. The two profile types occur with about equal frequency

in the wild *T. boeoticum*, but the domesticated derivative *T. monococcum* consists almost exclusively of the short type.

The gliadin pattern of specific diploid and tetraploid accessions show marked homology (Figs. 1 and 7; 2 and 11), lending credibility to the theory that the diploids contributed at least one genome to the tetraploids. Furthermore, the variability of the diploid gliadin pattern provides for the possible existence of complementing pairs, in effect, A- and B-genome types, which could account for specific tetraploid gliadin profiles as in the case of previously confirmed amphiploid parentages (16–18). Protein electrophoretic studies by G. Ladizinsky and B. L. Johnson (manuscript submitted for publication) show that tetraploids of *Avena strigosa* Schreb. comprise a diversity of profile types, some of which are identical with specific diploid profile types of the same species, and some of which can be simulated by complementing pairs of *strigosa* diploids.

Albumin profiles of the tetraploid wheats can be less readily accounted for by means of the present species collection, owing to the scarcity of prominent homologues in the diploid pattern. Alternatively, it could be argued that diploid types complementary for all of the tetraploid homologues might not be required. This assumes that two dimeric proteins of different migration velocity, each from a different parental diploid, could produce a series of albumin bands in the amphiploid by random reassociation of subunits. Nevertheless, very little evidence of such protein hybridization has been found among the ethanol seed extracts used in these studies.

More significantly, the band at −9.0 cm, which distinguishes the emmer from the *timopheevi* tetraploids, is, in fact, frequent in collections of the diploid wheats (Figs. 3 and 4) from eastern Anatolia. Recently, diploid types (Figs. 5 and 6) have been recovered to account for the tetraploid homologue at −9.7 and possibly that at about −8.4 cm. Furthermore, the albumin patterns obtained by increasing the concentration of the protein sample of selected accessions suggest that the diploid population harbors the genes required to account for some of the more complex tetraploid albumin profiles. At double the normal protein concentration, three diploid accessions (Figs. 42–44) show homologues of most of the bands falling between −6.8 and −9.7 cm in the tetraploids.



FIGS. 37–41. Electrophoretic profiles of seed proteins. FIG. 37. *Triticum boeoticum* accession G560. FIG. 38. *Aegilops bicornis* accession G365. FIG. 39. A mixture of protein (1:1) from accessions G560 and G365. FIG. 40. The amphidiploid from accessions G650 and G365. FIG. 41. *T. dicoccum* accession G497.

FIGS. 42–45. Electrophoretic profiles of seed proteins from samples of twice the normal protein concentration. FIGS. 42–44. *Triticum boeoticum* accessions G1169, G558, and G1546, respectively. FIG. 45. *T. dicoccum* accession G497 (normal protein concentration).

Morphological evidence

The remarkable similarity in spikelet morphology commented on by earlier investigators (5, 25) also indicates that the diploid wheats may well have contributed the B as well as the A genome to the tetraploids. Examination of the material listed in Table 1 confirmed the observation of Schiemann (25) that both the wild diploids and the wild tetraploids have three florets per spikelet of which a maximum of two are fertile, and that both consistently have two-keeled glumes. This examination also showed that both are essentially equally variable with respect to the relative length and width of the rachis internode. In the cultivated derivatives these three attributes, ostensibly modified by selection for high yield, seem to provide misleading evidence regarding the B donor. Thus, the tetraploid cultivars frequently have four or more florets per spikelet, three or more being fertile; their secondary keel is essentially eliminated; and their nonshattering rachis has a long slender internode. Such cultivars were included by Sarkar and Stebbins (7) in the extrapolation that pointed to a B parent with four florets per spikelet, glumes with a single keel, and long narrow rachis internodes.

Accessions of *T. monococcum* (G368, G1472), with a rudimentary fourth floret per spikelet, indicate that the diploid wheat can also adequately account for the increase in floret number observed in the tetraploid cultivars.

The observation, that the glume teeth of the diploids are subequal in length whereas the secondary tooth of the tetraploids is greatly reduced, was taken as evidence that the B parent had truncated glumes (7). However, the greater array of tetraploid types illustrated by Schiemann (25) clearly shows the essential difference between *T. boeoticum* and *T. dicoccoides* in this respect to be the greater size of the primary tooth in *T. dicoccoides* rather than the reduced size of the secondary tooth. The material now available (Table 1) confirms the variation in length of the primary tooth of *T. dicoccoides* illustrated by Schiemann, and shows that types with long to virtually awn-like primary teeth occur also in the diploid wheats albeit at a low frequency. For example *T. boeoticum* var. *virido-boeoticum* (G1735) and *T. boeoticum* var. *fuscum* (G559) from the U.S.S.R. collection have long primary teeth comprising up to one third of the length of the glume in the latter accession.

Cytogenetic evidence

Aegilops diploids are sufficiently genomically differentiated from the *Triticum* A genome to provide for preferential pairing in their amphiploids. Lack of evidence of comparable genomic differentiation within the diploid wheats presumably explains why they have been largely dismissed as a possible source of the B genome. To the knowledge of the writer, however, the great diversity of morphological types among the Transcaucasian diploid wheats has not been subjected to genome analysis.

Camera (5) speculated that in view of the distinct karyotypes observed within *T. monococcum*, much greater karyotypic differences might be expected among different diploid species of *Triticum*. Sarkar and Stebbins (7) suggested that the concept of A, B, and S as distinct genomes breaks down and that here we must deal with different degrees of homology.

The diploidizing gene on chromosome 5B that suppresses intergenomic pairing in polyploids and hybrids presumably has played a significant role in the differentiation of the two genomes of the tetraploid wheats. However, the degree of chromosomal or genic differentiation required between two emerging genomes before the 5B gene recognizes them as homoeologous rather than homologous is not known. Riley *et al.* (9) suggested that the 5B gene arose at the tetraploid level. However, a low frequency of such a gene in a diploid population could provide the mechanism to account for intraspecific tetraploid complexes such as that in *Avena strigosa* Schreb. (26) and that postulated here for the wild *Triticum* tetraploids.

The wild wheats of the Near East apparently still hold the answer to the question about the exact identity of the B donor.

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