Biodiversity and Wheat Improvement
Edited by A.B. Damania
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A Wiley-Sayce Publication

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Use of genome-specific antigens and prolamin electrophoresis in the evaluation of wheat and its wild relatives

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In recent decades seed proteins, because of their biological specificity, have been used extensively in research efforts aimed at solving many important problems in applied botany, genetics and plant breeding; these efforts include research on the origin of cultivated plants and the evaluation of their relationship with wild relatives. Proteins are well known as reliable markers of genes and the genetic structures with which these genes have structural and functional conjunction. They can also be used as a marker of a diploid species or a definite genome inside an allopolyploid's polygenome (Konarev, 1991).

For about a quarter of a century now, marking genetic systems (genes, chromosomes, genomes) and biological ones (varieties, biotypes, lines, populations) has involved the use of such protein characters as molecular multiplicity and polypeptide polymorphism, disclosed by electrophoresis, and antigenic properties of molecules, which undergo immunochemical evaluation by applying specific antiserums obtained for corresponding marker protein antigens. In the first case, the intraspecific (gene and allelic) variability is revealed, whereas in the second one, there is the possibility of identifying species and genomes, implementing genome analysis of allopolyploids and evaluating genomic relations between species in polyploid complexes.

To address the problems encountered in plant breeding in cereals, the most efficient and easily applied proteins are caryopsis endosperm proteins — prolamins and non-prolamin proteins of the alcohol fraction. Being the most multivalent and polymorphic proteins, prolamins are now widely used in electrophoretic identification of varieties and analysis of populations. Non-prolamin proteins appeared to be active antigens with distinctly expressed species and genome specificity (Konarev, 1979b; Konarev and Chmelev, 1986, 1990; Chmelev et al., 1991).

This chapter presents some results of the work which has been conducted at the Vavilov Institute in Russia on the analysis of relationships among species and genomes of wheat and related species by seed proteins.

GENOME-SPECIFIC SEED PROTEINS USED IN GENOMIC ANALYSIS OF CEREALS

To exploit the use of proteins as markers of genetic systems requires an understanding of the nature of these proteins and their functional affiliation. Plant proteins are, in general, good antigens. This may be explained by the remoteness of a plant organism from an animal one, which produces antigens against an alien protein. Sufficiently high antigenic activity is, in particular, characteristic of seed proteins, such as albumins and globulins. In cereal caryopses such proteins are represented by enzymes, enzyme inhibitors and membrane proteins. The membrane proteins, as has recently been discovered (Konarev and Chmelev, 1990), constitute the main part of highly active genome-specific antigens, which have been used at the Vavilov Institute to establish genome relationships in various cereals for over 20 years (Konarev, 1983). To identify the genome as a complex genetic system it is obviously preferable to possess a wider range of proteins from the genome (that is, from different chromosomes) as the reagents in immunochemical analysis, and a more complete population of immunoglobulins specific to these antigens. A peculiar concentrate of genome-specific proteins was a fraction of wheat albumins accompanying prolamins in the alcohol extract and known in the literature as the non-prolamin protein fraction.

From a series of experiments we conducted we concluded that the proteins which form the marker component in the precipitation spectrum (marker of the genome) were represented by a group of components with different electrophoretic mobility but similar antigenic properties. These components are controlled by different chromosomes of corresponding genomes (Konarev, 1979a, b, 1981a; Konarev and Chmelev, 1986), and thus antigens in precipitation spectra may be considered as markers of the genetic material of a genome or its major part. The heterogeneity of proteins with antigenic properties, identical to those of serological markers of *Triticum* L. and *Elytrigia* Desf. genomes and the albumin 0. 19 antigen marker, was shown by Konarev (1978, 1981a) and Konarev and Chmelev (1986).

A detailed examination of the nature of the analysed antigens, using procedures such as immunoaffinity chromatography and the application of monoclonal antibodies, showed that the most active genome-specific protein antigens of cereal seeds are lipoproteins of cell membranes (Chmelev et al., 1991). In this case, the genome level of antigenic specificity of these proteins is comprehensible, along with the clear coincidence of the results of our genome relationship research with those of cytogenetic analyses. Hydrophobic proteins (lypoproteins and glycoproteins) play an important role in specific interrelations between cells in an organism; a leading role is assigned to them in molecular mechanisms of 'recognition' as they are receptors. Thus, available data confirms the efficiency of applying genome-specific lipoproteins and several other seed proteins as antigen markers of cereal species and genomes.

GENOMES OF POLYPLOID WHEAT

In terms of their genome composition, the polyploid species of wheat belong to two evolutionary groups: *turgidum-aestivum*, with the genome formulae AABB and AABBDD; and *timopheevi*, with the genome formula AAGG. It has been assumed that wild einkorn wheat, *Triticum boeoticum* Boiss., is the source of genome A, whereas *Aegilops speltoides* Tauch. or another species of the *Sitopsis* section is the source of genome B. Bread wheat and its whole range of hexaploid forms originated from a tetraploid form which had received genome D from its diploid bearers, that is, representatives of *Ae. tauschii* Coss. (*Ae. squarrosa* L.). The problem of wheat genomes has been discussed by many workers

(Kihara, 1924; Sears, 1956; Riley and Bell, 1958; Konarev et al., 1976; Konarev, 1983), but remains unsolved.

On the basis of cereal genome analysis by protein markers, we are continuing to investigate the concept that wild einkorn *T. urartu* or another closely related form was the phylogenetic donor of genome A in the *turgidum-aestivum* group of wheat species, while *T. boeoticum* was the donor for the *timopheevi* group (Konarev et al., 1976; Konarev et al., 1979a; Konarev, 1983).

Konarev et al. (1974) were the first to publish information on the relationship (by origin) of the emmer wheat genome A to wild einkorn *T. urartu*. This was soon confirmed by Johnson (1975), although he associated it with genome B. Chapman et al. (1976) convincingly proved the first finding using cytogenetic methods; they showed that chromosomes of *T. urartu*, when hybridized with bread wheat, conjugate at meiosis only with chromosomes of genome A. Later, this was confirmed by improved immunochemical methods, using the above-mentioned marker antigens. The participation of *T. urartu* in the formation of wheat genome A was also reported by Dvorak et al. (1988) on the basis of data from wheat genome DNA restriction analysis. There is also indirect evidence of the homology of the *T. urartu* genome to the first genome of the *turgidum-aestivum* group (for example, by susceptibility to a series of fungal pathogens, by colour and shape of the caryopsis, and by leaf downiness).

The available information on the two types of genome A was used by Dorofeev et al. (1979) as the basis for subdividing the *Triticum* genus into subgenera *triticum* and *boeoticum* Migusch. et Dorof. It should be noted that the marker antigens of *T. urartu* represented in the *turgidum* group are significantly poorer than the antigens of *T. boeoticum* in the *timopheevi* group (Konarev, 1975). The latter are expressed to the fullest degree in *T. araraticum*, and to a lesser extent in *T. timonovum*, *T. zhukovskyi*, *T. fungicidum* and *T. timopheevi* (Konarev et al., 1971).

Wild emmer wheat samples were divided by genome A into two categories. One of them, which originated in Iraq, appeared to be identical to *T. araraticum* and carried genome A^b. The other, which originated in Syria, was identical to *T. dicoccum* and contained genome A^u (Konarev, 1975; Konarev et al., 1976).

More complex was the question of the origin of the second genome in polyploid wheats. By using serological markers, we found that *Ae. speltoides* could not have been the donor of genome B in the *turgidum-aestivum* group (Konarev et al., 1971). Also, einkorn wheats do not possess the specific antigens typical for genomes B and G. The proteins in the overwhelming majority of wheat species from the *turgidum-aestivum* group revealed antigens typical for the genome of *Ae. longissima*, while the proteins of wheat with genome G revealed antigens typical for *Ae. speltoides*. It seemed likely, therefore, that *Ae. speltoides* (genome B^{sp}) could be the source of genome G, whereas *Ae. longissima* or a closely related form could be the source of genome B^l, at least for separate forms of the *turgidum-aestivum* group (Konarev et al., 1979a).

In some forms and biotypes of *T. persicum*, antigens of genome B are poorly identified or are not identified at all (Barisashvili et al., 1979). They also do not have the specific antigens which mark genomes A^b and B^{sp}; that is, these samples do not belong to the species in the *timopheevi* group. However, minor antigens common for genomes B^l B^{sp} and D were discerned in *T. durum* and *T. araraticum*, respectively (*see* Table 1 *overleaf*). These findings and the existence of well-expressed antigen B^l in a sample of *T. persicum* suggest that this genome originates, if not from *Ae. longissima*, then from a closely related form. It is also possible that the antigen specific to genome B^l has been lost in the majority of *T. persicum* forms during evolution. This could be directly associated with the secondary origin of the species analysed.

Antigens of genome B^l are poorly represented in the proteins of most *T. dicoccum* biotypes; in some species (14 out of the 62 analysed) they were not found at all (Peneva, 1979). As proteins

Table 1 **Antigenic composition of genome-specific seed proteins of *Triticum, Aegilops, Elytrigia, Elymus* and *Agropyron* species[a]**

Species	2n	Antigens												
		A^b	A	A^b	A	B^1	D	B^1	B^{sp}	D	0.19	E	S	J
Triticum boeoticum	14	+	-	-	+	-	-	-	-	-	-			
T. urartu	14	-	+	+	-	-	-	+	-	-	-			
T. durum	28	-	+	-	+	-	+	+	-	-	-			
T. aestivum	42	-	+	-	+	+	+	+	-	-	-			
T. timopheevii	28	+	-	-	-	-	+	+	-	-	-			
T. araraticum	28	+	-	+	-	-	+	+	-	-	-			
Aegilops longissima	14	-	-	-	+	-	+	+	-	-	-			
Ae. tauschii str.	14	-	-	+	+	+	+	+	-	-	-			
Elytrigia elongata	56, 70	+	+	.	.	.	+	+	+	+	+	.	.	.
E. intermedia	42	+	+	-	-	-	+	+	+	+	-	.	.	.
E. trichophora	42	+	+	-	-	-	+	+	+	+	-	.	.	.
E. juncea	42	+	+	-	-	-	+	+	+	+	-	.	.	.
E. mucronata	42	+	+	-	-	-	+	+	+	+	-	.	.	.
E. scythica	28	+	-	.	.	.	+	+	+	+	-	+		
E. nodosa	28	+	-	+	+	+	+	+	-	+	-			
E. juncea	14	+	-	+	-	+	+	+	-	-	+			
E. elongata	14	+	-	.	.	.	-	-	+	-	+	-	-	
E. caespitosa	14	+	-	+	+	-	+	-	+	-	-			
E. stipipholia	14	+	-	.	.	.	+	-	+	-	-	+	-	
E. repens	42	+	-	+	-	-	+	-	-	+	-			
E. smithii	28,56	+	-	+	-	-	+	-	+	-	-			
Agropyron rechingeri	28	-	-	-	-	+	+	-	+	-	-			
Ag. striatum	28	-	-	-	-	+	+	-	-	-	-			
Ag. libanoticum	14	-	-	-	+	-	+	-	-	+	-			
Ag. yezoense	28	-	-	-	-	-	.	.	.	-	-	+	-	
Ag. cristatum	14, 28, 42	+	-	.	.	.	-	-	+	+	-	+	-	
Ag. desertorum	28	+	-	.	.	.	-	-	+	+	-	+	-	
Elymus caninus	28	.	.	.	-	+	-	+	+	-	+	+	-	
E. canadensis	28	-	-	+	-	+	+	-	+	+	-			
E. fibrosus	28	.	.	.	-	+	-	+	+	-	-	+	-	
E. triticoides	28	-	-	-	-	+	+	-	+	-	-			

Note: a + = antigen present, - = antigen absent, . = traces of antigen

homological to other relatives have not yet been found in these samples, it may be assumed that antigens B^1 are weak here, and the serological methods used for their identification not sensitive enough.

It should be noted that species of subsection *Emarginata* in section *Sitopsis (Ae. longissima, Ae. sharonensis* and *Ae. bicornis*) have sometimes been excluded from the list of possible donors of

genome B of *turgidum* wheats, mainly because of the small number of meiotic chromosome pairing in the wheat hybrids and species mentioned. The reason for this seems to be that species in the *Emarginata* subsection have a suppressor gene of homological pairing, acting together with gene *Ph* of polyploid wheat. Besides, Feldman (1978) reported that pairing frequency among polyploid wheat hybrids with *Ae. longissima* is many times higher than with *Ae. speltoides*. In addition, *Ae. longissima* rarely makes bivalents with genome A. Feldman (1978) suggests that the most likely donor of wheat genome B is *Ae. searsii*. This does not contradict our findings; according to the data derived from immunochemical analysis, *Ae. searsii* is identical to *Ae. longissima* and carries genome B^1 (Peneva and Konarev, 1982). *Ae. searsii* occupies the area in common with *T. dicoccoides* in the *turgidum* group and occurs in the populations of this species.

As far as genome D is concerned, judging by the antigenic composition of hexaploid wheat seed proteins, its most likely donor is *Ae. tauschii* ssp. *strangulata*, which carries genome D^{str} (Konarev et al., 1976, 1979a). This is confirmed by gliadin electrophoresis and some indirect data acquired by substituting genome D in several bread wheat cultivars with genomes of different *Ae. tauschii* biotypes (Kerber and Tipples, 1969; Konarev, 1983). For all this, the common and reliable marker of chromosome 1D, both for *Ae. tauschii* and all hexaploid wheat forms, is the duplex of electrophoretic components of omega-gliadin 89. In the alpha-zone of the spectrum, bread wheat biotypes and cultivars nearly always possess alpha-gliadin 6_1, coded by chromosome $6D^{st}$. Thus, five genomes have been identified by protein antigens in the *Triticum* genus; they are lettered A^u, A^b, B^1, B^{sp} and D^{str}, according to their origin.

Obviously, these data should be regarded only as one stage in the study of the nature and origin of polyploid wheat genomes. Final answers may never be found, for the following reasons:

- in the process of evolution, genomes could change as a result of their integration in the allopolyploid genotype and because of introgressions of genetic material from other species
- genuine donors of some wheat genomes could disappear

Synthesis of an amphidiploid from presumed donors has emerged as a radical way of proving the origin of this or that genome. However, each natural wheat amphidiploid is the result of the long and complex development of forms in certain conditions, the reproduction of which is practically impossible. For this reason, even with the presence of genuine genome donors, a researcher may rely only on an approximate similarity between a synthetic amphidiploid and the natural one.

It is not yet clear to what extent the type of cytoplasm affects the genome's expression, in particular the change of the plasmotype when shifting to a new level of ploidy. Although such influence cannot be doubted, it is difficult to checked as it has evolved over a long period.

Indirect support for the proposed scheme of wheat phylogenesis is as follows. Comparative analysis of species-specific antigens in the diploid possessors of genomes A and B showed that *Ae. speltoides* stands closest of all *Aegilops* species to einkorn *T. boeoticum* and *T. monococcum*, whereas *Ae. longissima* stands closest to einkorn *T. urartu* (Peneva and Migushova, 1973; Konarev et al., 1976). It may be assumed that such selective biological compatibility could be one of the pre-requisites for the formation of amphidiploids with two types of genome A and B combinations; that is, A^b-B^{sp} and A^u-B^l, which were the sources of the two evolutionary groups of polyploid wheat. It is possible that the same selective principle might have operated at an early stage of development of hexaploid wheat with the genome formula $A^u A^u B^l B^l D^{st} D^{st}$. Judging by the degree of homology between polyploid wheat genomes and their donors, the *timopheevi* group is younger than the *turgidum* one. Protein

'portraits' of the latter's relatives have, to a large extent, been erased (Konarev, 1975). Antigens of genome D are more clearly expressed in hexaploid wheat proteins than A^u and B^l antigens. This reflects the later origin of *T. aestivum* compared with the initial amphidiploid, which generated tetraploid wheat forms with the genome formula $A^u A^u B^l B^l$.

Erasing the initial genome portraits in the evolution of polyploid wheat took place as a result of chromosome transformations in the polygenome integration process and undoubtedly by introgressions, involving genetic material from other species and, initially, from the subtribe Triticinae. In this area, rye has undergone the most detailed study. Its spontaneous introgressions were found in many wheat varieties by a series of chromosomes (Driscol, 1983). We identified one of them (1B/1R) in bread wheat varieties and lines by the monomorphic triplex of electrophoretic components of secalin (rye prolamin) omega-234, coded by a cluster of genes from chromosome 1R (Konarev et al., 1979a). Introgressions have been reported from several *Aegilops* and *Agropyron* species. The possibility of introgressions points to homoeology and compatibility of genomes belonging to different genera by definite loci. A criterion of such compatibility may be the availability of common species-specific protein antigens in the species. In this respect, the genera *Elytrigia, Elymus, Agropyron* and *Leymus* are of great interest as possible participants in polyploid wheat genome formation and as potential sources of wheat improvement.

ANALYSIS OF *TRITICUM, ELYTRIGIA, ELYMUS* AND *AGROPYRON* USING GENOME-SPECIFIC PROTEIN ANTIGENS

Like the *Triticum* and *Aegilops* genera, *Elytrigia, Agropyron* and *Elymus* include species of different ploidy levels: 2x, 4x, 6x and more with the number of chromosomes 2n = 14, 28, 42, 56, etc. Bozzini et al. (1973) was the first to demonstrate a close immunochemical relationship between bread wheat and some species of these cereals, based on water-soluble seed proteins. Later, we undertook detailed investigations using monospecific immune serums on the following protein antigens of wheat and *Aegilops* (*see* Table 1):

- *Antigen* A^b: Typical for seed proteins of diploid wheat *T. boeoticum* and *T. monococcum*; found in seed proteins of polyploid wheat of the *timopheevi* phylogenetic group (Konarev et al., 1971; Konarev, 1975)
- *Antigen* A^u: Typical for seed proteins of diploid wheat *T. urartu*; identified in seed proteins of polyploid wheats of the emmer phylogenetic group and hexaploid wheats with genome D, *T. aestivum* and others (Konarev et al., 1974; Konarev, 1975)
- *Antigen* A^bA^u: Typical for seed proteins of all diploid wheats; also found in proteins of some wild tetraploid wheats (Konarev, 1975; Barisashvili et al., 1979)
- *Antigen* B^l: Typical for seed proteins of diploid *Ae. longissima*; also found in polyploid emmer wheat proteins and hexaploids with genome D (Peneva and Migushova, 1973; Konarev et al., 1976a; Peneva, 1979)
- *Antigen D*: Typical for diploid *Ae. tauschii* proteins; identified in hexaploid wheat proteins (Konarev et al., 1976, 1979a)

- *Antigen B^l B^{sp} D*: Two antigens, common for *Aegilops* species and polyploid wheats; absent in diploid wheat proteins (Konarev et al., 1979b)
- *Antigen albumin 0.19*. Typical for seed proteins of all *Triticum* and *Aegilops* species except *T. boeoticum* and *T. monococcum* (Konarev, 1978).

The results of the immunochemical analysis presented in Table 1 show that several *Elytrigia* species, including *E. elongata* (2n = 56, 70), *E. intermedia*, *E. trichophora*, *E. mucronata* and *E. juncea* (2n = 42), possess antigens in common with *T. aestivum* by all three genomes (A, B and D). Antigen 0.19 is also typical for them. Other *Elytrigia* species have common antigens only as far as one of these genomes is concerned. Antigens in common for genome A are present in *E. elongata* (2n = 14), *E. repens* (2n = 42) and *E. smithii* (2n = 28, 56); for genome D, in the tetraploid wheatgrasses *Agropyron subsecundum* Link., *Ag. rechineri*, *Ag. pringlei* Hitch., *Ag. striatum*, *E. elongata* (Host.) Nevski (2n = 28); for genomes B^l and A^b, in *E. caespitosa* (Koch) Nevski (2n = 14), *E. stipifolia* (Czern. ex Nevski) Nevski (2n = 14); and for genome B^l, in *Ag. libanoticum* Hack. ex Kneuck (2n = 14).

A series of *Elytrigia* and *Agropyron* species (except *Ag. cristatum* and *Ag. desertorum*, the crested wheatgrasses) do not have wheat genome antigens or have the most common ('ancient') antigens of the B^lB^{sp} D type. Such species include *E. ferganensis*, *E. scabriglume*, *Ag. inerme*, *Ag. spicatum*, *Ag. latiglume* and *Ag. yezoense* (Konarev et al., 1979b). It appears that representatives of these species do not cross, or cross poorly, with wheat species. A small degree of homology between genomes of *T. aestivum* and *Ag. yezoense* was demonstrated by Sharma and Gill (1983). Crossing ability between other *Elytrigia* species and wheat corresponds with the presence of protein antigens marking genomes A, B^l and D in these species (that is, genomes of einkorn *Ae. longissima* and *Ae. tauschii*) (Sakamoto, 1973; Tsitsin, 1978).

The antigen marking genome D is distinctly identified in the proteins of all members of the *Elymus* genus except for *E. dachuricus* Turez. ex Griseb. (2n = 28). It is extremely poorly represented (often absent) in *Ag. cristatum* and *Ag. desertorum*. However, the antigens specific to the einkorn genome are well revealed here. The data we have obtained enable us to conclude that *Elymus* species stand closer to *T. aestivum* for genome D, but to *Agropyron* species for genome A, as shown in Table 1.

An important protein character for measuring mutual relationships between wheat forms with genome B^l is antigen 0.19, extracted from *T. aestivum* seed. The use of double immunodiffusion and quantitative immunoelectrophoresis showed that this antigen's expression intensity is a good indication of the degree of genetic compatibility of the analysed species and forms with *T. aestivum*. Thus, it is absent in proteins of all *Hordeum* species and of *Elytrigia* species which do not cross with bread wheat. Antigen 0.19 is poorly expressed in proteins of the *timopheevi* wheat group and *Secale* species, and best expressed in *Triticum* species of the *turgidum-aestivum* phylogenetic group (Konarev, 1978).

'Own' genome-specific antigens of wheatgrass which have been identified in proteins of diploid species are:

- *Antigen E*: Typical for diploid *E. elongata* (Konarev, 1979a, b; Konarev et al., 1979b; Konarev, 1981a)
- *Antigen S*: Typical for seed proteins of diploid *E. stipifolia*, *E. ferganense*, etc. (Konarev et al., 1979b; Vasiljeva, 1979; Konarev, 1981a)
- *Antigen J*: Typical for seed proteins of diploid *E. juncea* (Konarev, 1981b)

Antigens marking genome E have been found in proteins of 28-, 56- and 70-chromosome *E. elongata* races, as well as in proteins of *E. smithii* Rybd. (2n = 28, 56), *Ag. littorale* (Host.) Dum (2n = 28), *Ag. rechingeri* (2n = 28) and several *Elymus* species. In proteins of *Agropyron* species, including *Ag. cristatum* and *Ag. desertorum*, antigens of genome E are absent, as shown in Table 1.

Antigens marking genome S have been found in most *Elytrigia* species, almost always where there are no antigens of genome E. These antigens are in the proteins of all *Agropyron* species and many *Elymus* species. It should be noted that genome S differentiates *Elymus* species into two groups. On the basis of their antigenic composition of alcohol fraction proteins, *Elymus* species with genome S stand closer to *Elytrigia* species possessing genome S than to the species of their own genus which do not have this genome (Konarev and Vasiljeva, 1979). *Elymus* species in which the proteins contain antigens of genome S cross well with diploid wheatgrass species carrying this genome. All this agrees with the results of cytogenetic analysis (Sakamoto, 1973) and support the opinion of those researchers (Dewey, 1974; Jaaska, 1974; Tsvelev, 1976) who suggest that only the species with genome S should be attributed to the *Elymus* genus. It has also been suggested that only those forms which cross with wheat should remain in the *Elytrigia* genus (Tsitsin, 1978; Konarev, Vasiljeva, 1979). A similar classification, based on the data of caryological and cytogenetic genome analysis, was proposed by Sakamoto (1973), whereby the wheatgrass species which could be crossed with wheat were separated from *Agropyron* Gaertn. Although an antigen of genome S has been found in the latter, in terms of antigenic composition of seed proteins they differ from *Elytrigia* and *Elymus*, which contain this genome.

Apart from *Elytrigia juncea* (2n = 14, 28), antigens of genome J have been identified as traces in 42-chromosome *E. juncea* as well as in polyploid forms of *E. elongata* (2n = 56, 70), *E. intermedia* (Host.) Nevski (2n = 42) and *E. trichophora* (Link) Nevski (2n = 42) (*see* Table 1). Genome J unites wheatgrass species, closely related by their antigenic composition, to wheat species, allowing easy crossability (Konarev, 1981b).

Thus, judging by protein antigens, the diploid *Agropyron*, *Elytrigia* and *Elymus* species carry genomes E, S and J. In most cases they show similarity to wheat genomes either by einkorns (A^bA^u) or by *Aegilops* (B^l, D). Polyploid species may involve, together with the named genomes, the genetic material from 'wheat' genomes. As far as such wheatgrass species and chromosome races are concerned, they can be more distinctly defined as *E. elongata* (2n = 56, 70), *E. juncea* (2n = 28, 42), *E. trichophora* (2n = 42) and *E. intermedia* (2n = 42).

Genetic proximity and similarity in the genome organization of wheat and several wheatgrass species is indicated not only by their high homology regarding species-specific antigens but also by composition and properties of seed storage proteins. Such similarity is the closest between wheat and polyploid wheatgrass species. The latter have a 'wheat-like' type of prolamin electrophoretic spectrum structure. Moreover, prolamins of the wheatgrass forms, most closely related to wheat, contain a monomorphic duplex of components omega-89, marking chromosome 1D of *Ae. tauschii* and *T. aestivum*. It is also characteristic for gliadins of *Ae. umbellulata* (C^uC^u), *Ae. comosa* (MM) and all polyploid *Aegilops* species including genomes D, C^u or M (Konarev, 1983). We have shown that gliadins omega-89 were conjugated with the yield of gluten and its elasticity. Such conjugation is also evident in wheatgrasses. Their polyploid species, having a 'wheat-like' structure of gliadin spectrum, are capable of yielding elastic gluten at a rate of 60% from the flour of some forms, which is much higher than in baking wheat.

The above-mentioned data on genome relations and origin have been obtained using serological methods, including double immunodiffusion. The latter has many advantages but it also has several limitations. For example, it does not enable one to make an objective evaluation of such characteristics of genome-specific proteins as their quantitative content and composition of components. We have

shown (Konarev, 1979b, 1981a) that these parameters are the ones of fundamental importance in determining the degree of relationship between cereal species and genomes during genome analysis by antigen markers; a genome carrier (donor) always possesses the most intensive component in the precipitation spectrum and the largest number of the components of corresponding genome-specific proteins during electrophoresis (Konarev and Chmelev, 1986, 1990).

At present, rapid testing and determination of the composition and quantitative content and antigens can be achieved using solid phase enzymoimmunoassay — enzyme-linked immunosorbent assay (ELISA) and immunoblotting. In the recent years, the Vavilov Institute has managed to develop a method of applying these techniques to genome-specific antigens of Triticeae (Chmelev et al., 1991). The data derived from enzymoimmunoassay correspond well with the results of double immunodiffusion, which involved visual determination of antigenic quantitative content by the intensity of the component in the precipitation spectrum. All this enhances the possibility of a more efficient genome analysis by protein antigens and of solving the problems faced in the search for markers of valuable qualities and characters of cereals.

PARTICIPATION OF TRITICEAE SPECIES IN THE FORMATION OF POLYPLOID WHEAT GENOMES

Genomes of polyploid wheat species have undergone evolutionary changes and have been losing their similarity to the initial genomes of diploid species. This has happened as a result of their integration in the polygenome (which is usually accompanied by, for example, various transformations in the form of inter-chromosome and inter-genome translocations, deletions, and heterochromatization of separate chromosome areas) and through introgressions of genetic material from other species. It is likely that members of the Triticinae subtribe were introgression sources; that is, *Aegilops* species, wheat grasses and rye, especially those species that have antigens in common with wheat and hence a common or similar principle of gene and chromosome organization of genomes. One of the criteria of such similarity may be the type of prolamin electrophoretic spectrum and the type of localization of their gene clusters in chromosomes (Konarev, 1991).

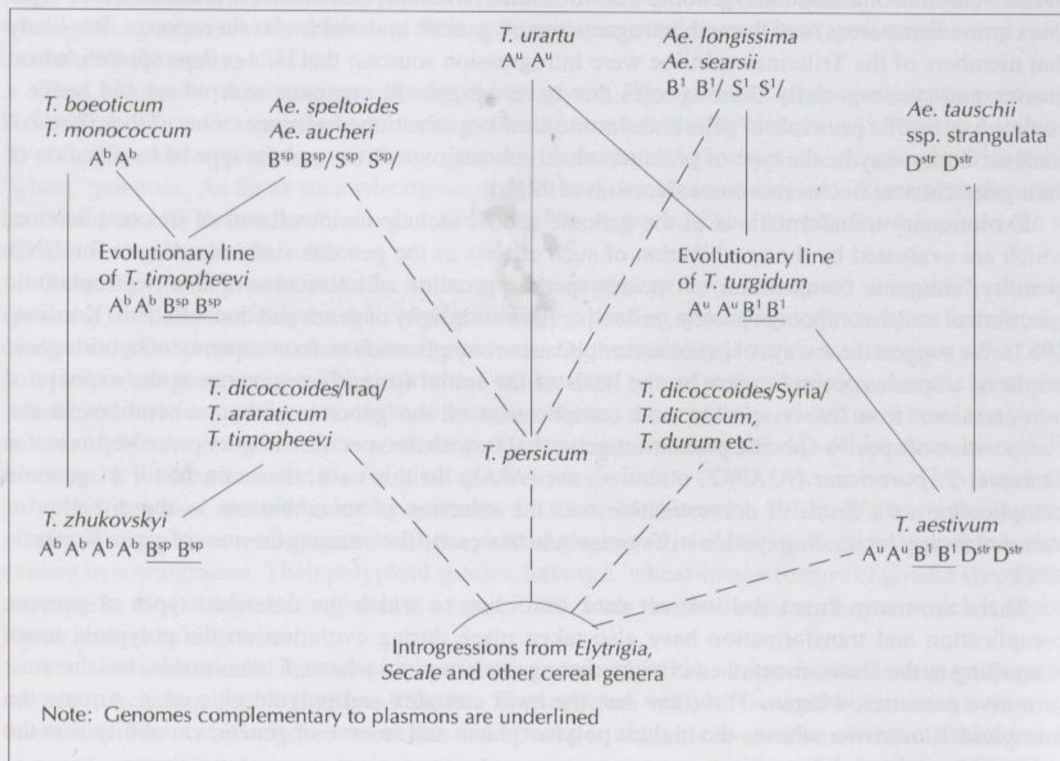
Evolutionary transformations of the genome should include various forms of its complication, which are evaluated by the combination of such criteria as the genome size according to the DNA quantity, antigenic composition of species-specific proteins and structure of the electrophoretic spectrum of multi-component protein, reflecting the multiplicity of genes and their allelism (Konarev, 1983). We suggest three ways of genome complication: complication as a consequence of hybridogenic origin of a species; complication on the basis of the initial (primitive) genome at the expense of introgressions from other species; and complication of the genome without alterations in the composition of species-specific protein antigens; that is, with the specific category preserved as in, for example, *T. boeoticum* (A^bA^b)-*T. monococcum* (A^bA^b). In this case, the main factor of genome complication as a result of domestication was the selection of recombinants in the direction of accumulation of loci coding synthesis of storage substances and determining the mass of grain (Konarev, 1983).

There are many direct and indirect data, according to which the described types of genome complication and transformation have also taken place during evolution on the polyploid level. According to the above-mentioned criteria, among the tetraploid wheats *T. dicoccoides* has the most primitive genomes, whereas *T. durum* has the most complex and polymorphic ones. Among the hexaploid *T. aestivum* wheats, the highest polymorphism and reserve of genetic variability is in the cultivated species *vulgare*.

Deep genome and chromosome transformations take place in allopolyploids during remote hybridization. In this respect, the transformations in experimental secondary hexaploid and, in particular, tetraploid triticale forms on the basis of octoploid ones are of special interest, being regarded as a model of evolutionary genome complication in the amphidiploid genotype (Peneva et al., 1986). A variant of such transformation may be presented in a simplified form as AABBDDRR—$AABBR_D R_D$. The third genome of secondary triticale is formed in accordance with homoeological substitution of the chromosomes of two genomes: genome D of wheat and genome R of rye, with the formation of segmental genome R_D. It is possible that hybridogeneity in allopolyploid species of related cereals by separate genomes has been formed following the same pattern as, for example, that in wheat and the wheatgrass species. It is worth noting that the method of differential staining has shown that the chromosomes of one of the three genomes of *Ag. intermedium* have a banding pattern similar to the banding pattern of the chromosomes of genome B in bread wheat (Piao Zhensan, 1982). It was not by chance that botanists included wheat genomes into wheatgrass species' genome formulae (Vakar, 1935).

In genome transformations and the realization of their potential in the morphogenesis of a polyploid plant, a very important role is assigned to plasmon. Judging by the data produced by many researchers, all forms of polyploid wheat obtained cytoplasm from *Sitopsis* group species (cytoplasm S). In line

Figure 1 The nature of genomes in *Triticum* and *Aegilops,* derived from data from genome analysis using protein markers



Note: Genomes complementary to plasmons are underlined

with our findings on the nature of wheat genomes, the plasmon of the *turgidum-aestivum* species is complementary to genome B^{l}, whereas the the plasmon of the *timopheevi* species is complementary to genome B^{sp}. To indicate the nature of the plasmon, we have suggested that a complementary genome should be underlined in an amphidiploid genome formula; thus, $A^{u}A^{u}B^{l}B^{l}$ for *turgidum-aestivum* and $A^{b}A^{b}B^{sp}B^{sp}$ for *timopheevi* (Konarev, 1988) (*see* Figure 1). Naturally, in the process of evolution the initial plasmon as a genetic system of the specific category co-adapted with polygenomes of different species and forms of polyploid wheat, and is now represented by the plasmotype variants corresponding to these species.

CONCLUSION

An analysis of *Triticum* and *Aegilops* sepcies, performed by species-specific protein antigens and reinforced by electrophoresis of multivalent monomorphic proteins, made it possible to identify five genomes in wheat, namely: genome A^{u} from *T. urartu* or closely related forms; genome A^{b} from *T. boeoticum*; genome B^{l} from *Ae. longissima, Ae. searsii* or *Ae. sharonensis*; genome B^{sp} from *Ae. speltoides* or *Ae. aucheri*; and genome D^{st} from *Ae. tauschii* ssp. *strangulata*. Genomes A^{u} and B^{l} are typical of the *turgidum-aestivum* wheat group, genomes A^{b} and B^{sp} are typical of the *timopheevi* group, and genome D^{str} is typical of the hexaploid *T. aestivum* wheat, shown in Figure 1.

In the process of evolution, genomes of polyploid wheat species have changed, not only through intra-genome transformations and inter-genome exchanges but also because of introgressions and the formation of hybridogenic (seg-mental) genomes. Identification of introgressions by protein markers shows that many related cereals participated in modern wheat genome formation and, consequently, in the evolution of the *Triticum* genus; among these were *Aegilops, Agropyron, Elymus, Elytrigia* and *Secale*. A special role in this process was played by the *Aegilops* genus; the development of the polyploid complex of wheat took place not only with the participation of separate *Aegilops* genomes, but also on the basis of its plasmotype and allopolyploid way of evolution.

The transformation of wheat genomes under the influence of cultivation occurred mainly through enlargement of a genome, resulting from the accumulation of genetic loci and systems, which provide a high level of genetic variability in commercial characters, including quantitative ones (productivity per ear, caryopsis size, endosperm volume and share of reserve nutritive components in the yield).

Data on the nature and origin of the genomes of wheat and its relatives have provided the basis for a new stage of investigation on the phylogeny and systematics of *Triticum* (Dorofeev et al., 1979). They offer new opportunities in the search for initial material to use in breeding for yield quality, immunity and resistance to stresses (Krivchenko et al., 1976). Widening and mobilizing the wheat genepool at the expense of its relatives by allopolyploid and introgression breeding will be assisted by the use of methods of genome and genetic analyses based on protein and other molecular markers.

Discussion

L.A. Morrison: Can you tell me what *Triticum fungicidum* Zhuk. looks like?

A.V. Konarev: It is a very interesting artificial amphiploid of *T. timopheevi* x *T. persicum (T. carthlicum)* and morphologically intermediate between the parents. It has a very high disease resistance.

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