Theor. Appl. Genet. 56, 273-284 (1980)

Electrophoretic Survey of Seedling Esterases in Wheats in Relation to Their Phylogeny

V. Jaaska
Institute of Zoology and Botany, Academy of Sciences of the Estonian SSR, Tartu, Estonian SSR (USSR)

Summary. Evolutionary and ontogenetic variation of six seedling esterases of independent genetic control is studied in polyploid wheats and their diploid relatives by means of polyacrylamide gel electrophoresis. Four of them are shown to be controlled by homoeoallelic genes in chromosomes of third, sixth and seventh homoeologous groups.

The isoesterase electrophoretic data are considered supporting a monophyletic origin of both the primitive tetraploid and the primitive hexaploid wheat from which contemporary taxa of polyploid wheats have emerged polyphyletically and polytopically through recurrent introgressive hybridization and accumulation of mutations. Ancestral diploids belonging or closely related to *Triticum boeoticum, T. urartu, Aegilops speltoides* and *Ae. tauschii* ssp. *strangulata* are genetically the most suitable genome donors of polyploid wheats. Diploids of the Emarginata subsection of the section Sitopsis, *Aegilops longissima* s.str., *Ae. sharonensis, Ae. searsii* and *Ae. bicornis*, are unsuitable for the role of the wheat B genome donors, being all fixed for the esterase B and D electromorphs different from those of tetraploid wheats.

Key words: Wheat esterases – Wheat phylogeny – Esterase isoenzymes – *Triticum* L. – *Aegilops* L.

1 Introduction

Carboxylesterases (EC 3.1.1.1) comprise a group of widely distributed hydrolytic enzymes capable of splitting carboxyl esterase bonds in various organic compounds. We have described polyacrylamide gel electrophoretic patterns of seedling esterases in wheat species in relation to phylogeny and ontogenetic regulation during germination in several publications (Jaaska 1969, 1971, 1974, 1976). Similar comparative studies of seed esterases in wheats utilizing polyacrylamide gel electrophoretic (Bhatia 1968; Mitra and Bhatia 1971) or isoelectric focusing techniques (Bozzini et al. 1973; Cubbadda et al. 1975; Nakai et al. 1969; Nakai 1973) have been published. Chromosomal control of wheat esterases has been studied by several authors (Barber et al. 1968; May et al. 1973; Nakai 1976).

The aim of the present paper is to comprise the polyacrylamide gel electrophoretic data about seedling esterases in polyploid wheats and their diploid relatives we have accumulated up to date.

2 Materials and Methods

2.1 Plant Material

The number of accessions studied is indicated in brackets.

1) Hexaploid wheats (*Triticum aestivum* L. s.l.): *T. spelta* L. (23); *T. macha* Dek. et Men. (11); *T. aestivum* L. s.str. (74); *T. compactum* Host (93); *T. sphaerococcum* Perc. (7). The chromosomal location of genes coding for esterase isoenzymes in polyploid wheats has been studied with the use of a series of nulli-tetrasomic and ditelocentric chromosome lines of *T. aestivum* cv. Chinese Spring.

2) Tetraploid wheats of the emmer group comprising *T. turgidum* L.s.l. and involving the cultivated *T. turgidum* L.s.str. (19); *T. durum* Desf. (42); *T. turanicum* Jakubz. (1); *T. aethiopicum* Jakubz. (14); *T. polonicum* L. (8); *T. jakubzinerii* Udacz. (1); *T. ispahanicum* Heslot (1); *T. carthlicum* Nevski (16), syn. *T. persicum* Vav. ex Zhuk. 1923, non Aitch. et Hemsl. 1888; *T. dicoccon* (Schrank) Schuebl. (44); *T. karamyschevii* Nevski (4), syn. *T. dicoccon* ssp. *georgicum* (Dek. et Men.) Flaksb., = *T. dicoccon* var. *chwamlicum* Supat. and the wild emmer *T. dicoccoides* (Koern.) Aaronsohn (26).

3) Tetraploid wheats of the timopheevii group (*T. timopheevii* Zhuk. s.l.), including the wild-growing *T. araraticum* Jakubz. (36) and the cultivated *T. timopheevii* Zhuk. s.str. (5).

4) Diploid wheats: *T. monococcum* L. s.l., including the cultivated *T. monococcum* L. s.str. (23) and the wild-growing *T. boeoticum* Boiss. s.l. (94), involving both the one-awned var. *'boeoticum'* and the two-awned var. *'thaoudar'*; *T. urartu* Thum. ex Gandilian (19).

0040-5752/80/0056/0273/$ 2.40

5) Diploid goatgrasses: *Aegilops tauschii* Coss. (110), syn. *Ae. squarrosa* auct., non L.; *Ae. speltoides* Tausch s.l. (49), involving the awnless var. *'speltoides'* (25), syn. *Ae. aucheri* sensu Zhuk. 1928, non Boiss. 1844, and the awned var. *'ligustica'* Bornm. (24), syn. *Ae. ligustica* Cosson, = *Ae. speltoides* auct., non Tausch; *Ae. mutica* Boiss. (5), syn. *Amblyopyrum muticum* (Boiss.) Eig; *Ae. bicornis* (Forsk.) Jaub. et Sp. (7); *Ae. longissima* Schweinf. et Muschl. s.str. (8); *Ae. sharonensis* Eig (9), *Ae. searsii* Feldman et Kislev (5); *Ae. caudata* L. (5); *Ae. umbellulata* Zhuk. (7); *Ae. comosa* Sibth. et Sm. (5).

2.2 Biochemical Methods

Enzyme extracts were prepared from the coleoptile, primary leaf or root tissues of individual four- to eight-days-old etiolated seedlings and subjected to electrophoresis in 10 per cent polyacrylamide gel slabs to stain for esterase activity as described previously (Jaaska 1974, 1976). The isoesterase bands are designated by unified distances of migration (D_m) from the origin to the anode expressed in arbitrary units on the scale at the left side of the figure. The isoesterases known or supposed to be encoded by separate gene loci are designated by capital letters (esterases A, B, C, etc.), and the corresponding gene loci – by arabic numbers (*Est-1*, *Est-2*, *Est-3*, etc.). The electrophoretic variants (alloesterases, homoeoallelic isoesterases of polyploids, hybrid isoenzymes) and the corresponding alleles will be labeled by numerical superscripts indicating the D_m values, the superscript 'O' specifying the absence of the esterase band. The underscripts specify wheat genomes (A, B, D) or chromosomes (3A, 6A, etc.) in which the esterase genes are located.

3 Results

3.1 Polyploid Wheats

3.1.1 Esterase A

is well-distinguished on the polyacrylamide gel electrophoretic enzymograms of polyploid wheats by the triplet phenotype with three fastest-moving isoesterases, $A^{5.0}$, $A^{4.75}$ and $A^{4.5}$. These isoesterasrs were shown (Barber et al. 1968) to be encoded by homomeric genes (homoeoalleles according to Washington, 1971), located on chromosomes of the third homoeologous group of the three wheat composite genomes, A, B and D. The isoesterase $A^{5.0}$ is controlled by homoeoalleles of chromosomes 3B and 3D, $Est\text{-}1_B$ and $Est\text{-}1_D$, whereas the isoesterase $A^{4.5}$ is specified by the 3A homoeoallele ($Est\text{-}1_A$). The data of the enzymograms 8-12 in Figure 1 essentially support this interpretation.

The same three-banded phenotype of esterase A was found to be inherent to all taxa and to most accessions of polyploid wheats, hexaploids and tetraploids, wild and cultivated. Three types of variation of esterase A was observed in polyploid wheats.

Among hexaploid wheats, all the accessions of *T. compactum* var. *'rubriceps'* of different geographic origin (Transcaucasia, Central Asia) revealed an exceptional five-banded phenotype of esterase A (3-4, Fig. 1) with three fast-moving bands, $A^{5.2}$, $A^{5.1}$ and $A^{4.9}$, present in addition to $A^{4.5}$ and $A^{4.75}$.

The only mutant phenotype in *T. dicoccoides* was that which lacked isoesterase $A^{5.0}$ specified by the B genome homoeoallele, as well as the hybrid isoesterase $A^{4.75}$, and showed the presence of only isoesterase $A^{4.5}$ of the A genome homoeoallele. This phenotype (2-3, Fig. 2) was characteristic of four accessions *T. dicoccoides*, and it was revealed in all tissues of seedlings (roots, primary leaf, coleoptile) of these accessions. Evidently, a mutational inactivation of the B genome homoeoallele of esterase A is fixed in these accessions of *T. dicoccoides*.

The one-banded esterase A phenotype was not ob-

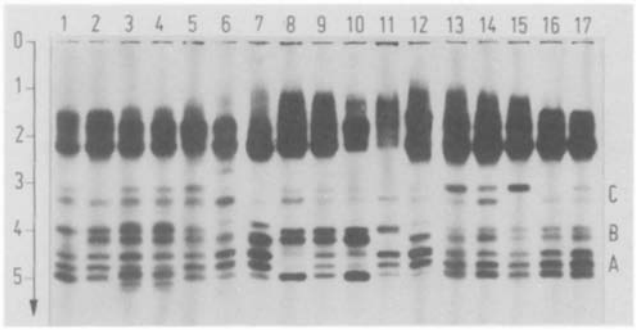

Fig. 1. Enzymograms of coleoptile esterases in polyploid wheats – variation and chromosomal control: 1 = *T. sphaerococcum* var. *rotundatum* from Pakistan; 2 = *T. sphaerococcum* var. *kibraicum* from India; 3-4 = *T. compactum* var. *rubriceps* from Turkey (3) and India (4); 5 = *T. spelta* var. *duhamelianum* from Switzerland; 6-7 = *T. dicoccon* from Turkey (6) and France (7); 8-17 = ditelocentrics and nulli-tetrasomics of *T. aestivum* cv. 'Chinsese Spring'; 8 = ditelo-3Aα; 9 = ditelo-3BL; 10 = nulli-3A/tetra-3B; 11 = nulli-3B/tetra-3A; 12 = nulli-3D/tetra-3A; 13 = nulli-7A/tetra-7D; 14 = nulli-7A/tetra-7B; 15 = nulli-7B/tetra-7D; 16 = nulli-7D/tetra-7A; 17 = ditelo-7DS. The origin is at the top and the anode is at the bottom

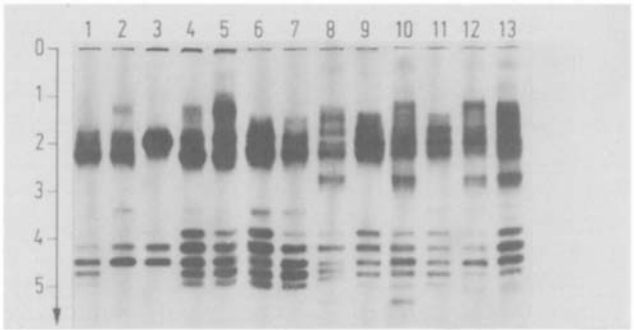

Fig. 2. Enzymograms of coleoptile esterases in tetraploid wheats: 1-4 = *T. dicoccoides* from Israel; 5 = *T. dicoccon* from France; 6 = *T. dicoccoides* from Iraq; 7-8 = *T. araraticum* from Turkey (7) and Iraq (8); 9 = *T. dicoccoides* from Iraq; 10 = *T. araraticum* from Iraq; 11 = *T. dicoccoides* from Turkey; 12 = *T. araraticum* from Iraq; 13 = *T. araraticum* from Turkey. The origin is at the top and the anode is at the bottom

served in cultivated emmers or in the *timopheevii*-group tetraploids. Two mutant phenotypes of esterase A with more closely spaced triplets than ordinarily (8, Fig. 2) were characteristic of some accessions of the wild tetraploid *T. araraticum*. In these mutant phenotypes, the esterase A band specified by the B genome homoeoallele has two electrophoretic variants with slightly lower mobilities instead of the isoesterase $A^{5.0}$.

No electrophoretically detectable variation of the A genome homeoallele has been recovered, the isoesterase $A^{4.5}$ being invariantly present in all accessions and taxa of polyploid wheats.

3.1.2 Esterase B

has been specified (Jaaska 1976) as a coleoptile-specific enzyme of slightly lower electrophoretic mobility than esterase A. It is represented in hexaploid wheats by three isoenzymes, $B^{4.2}$, $B^{4.0}$, and $B^{3.9}$ (1-5, Fig. 1), which exhibit highest activity in developing coleoptiles of young 3-4-day-old seedlings. Their activity and staining intensity on enzymograms decreases with the seedling age, band $B^{3.9}$ disappearing first followed by $B^{4.0}$ and lastly by band $B^{4.2}$.

The isoenzyme $B^{4.2}$ was lacking in nulli-3B/tetra-3A (11, Fig. 1) but it was present in ditelo-3BL (9, Fig. 1). This result suggests that $B^{4.2}$ is controlled by the B genome homoeoallele (*Est-2*$_B$) located on the long arm of the chromosome 3B. Band $B^{4.0}$ was lacking on the enzymogram of nulli-3D/tetra-3A (12, Fig. 1), but it was present in ditelo-3Dα. This implies that $B^{4.0}$ is specified by the D genome homoeoallele (*Est-2*$_D$) located in 3Dα. The slowest isoenzyme, $B^{3.9}$, which is faintly stained on the enzymograms of young coleoptiles of hexaploids, is controlled by the A genome homoeoallele (*Est-2*$_A$).

A noticable increase in the intensity of band $B^{4.2}$ on the enzymogram of nulli-3A/tetra-3B (10, Fig. 1) and the appearance of band $B^{3.9}$ on the enzymogram of older nulli-3D/tetra-3A coleoptile (12, Fig. 1) exemplify the gene dosage effect on the biosynthesis of esterase B isoenzymes in the hexaploid wheat.

All linneons of hexaploid wheats comprising *T. aestivum* s.l. exhibit the same three esterase B isoenzymes in common, with no variation in their electrophoretic mobility. One distinct mutant phenotype with the B genome isoesterase $B^{4.2}$ lacking has been recorded in hexaploids. It was frequent among the accessions of *T. macha* and *T. sphaerococcum* but was not found in *T. spelta* and was rare in *T. compactum*. Isoenzyme $B^{4.2}$ was also frequently lacking in accessions of *T. aestivum* s.str. originating from Pakistan and India, but it was expressed in most accessions of this hexaploid originating from Transcaucasia, Afghanistan, Europe and China.

No case of null-phenotype of *Est-2*$_D$ lacking $B^{4.0}$ has been recorded in hexaploid wheats.

Tetraploid wheats of the emmer group comprising *T. turgidum* s.l., including all linneons of cultivated emmers and wild-growing *T. dicoccoides*, are characterized (Fig. 2) by an invariant two-banded phenotype of esterase B, combining isoesterases $B^{4.2}$ and $B^{3.9}$ shown above to be encoded by the B and A genome homoeoalleles, respectively. $B^{4.2}$ predominates in activity, especially in older coleoptiles.

The same two-banded phenotype of *Est-2* with isoesterases $B^{4.2}$ and $B^{3.9}$ was also inherent to tetraploid wheats of the *timopheevii* group, the cultivated *T. timopheevii* s.str. and the wild *T. araraticum* (Fig. 2). No variation in the electrophoretic mobility of the two homoeoallelic isoesterases was found in tetraploid wheats. The only mutant phenotype was that lacking band $B^{4.2}$ which was recorded in three cases, for two accessions of *T. dicoccoides* and for one accession of *T. dicoccon*. The absence of hybrid isoenzymes in tetraploid wheats, showing codominant expression of both homoeoallelic genes of esterase B, implies its monomeric structure.

The recorded very rare occurrence of the silenced *Est-2*$_B^Q$ in the cultivated emmers contrasts its frequent appearance in some linneons of hexaploid wheats. This evidence suggests that the silencing of *Est-2*$_B$ has recurrently occurred on the hexaploid level, more frequently in some linneons and in certain geographic regions.

3.1.3 Esterase C

follows esterase B towards lower electrophoretic mobility on the coleoptile enzymograms (Fig. 1) and is characterized by a two-banded phenotype in hexaploids with isoesterases $C^{3.4}$ and $C^{3.15}$ found to be controlled by chromosomes of seventh homoeologous group. $C^{3.4}$ is lacking in nulli-7B/tetra-7D (15, Fig. 1) but is present in ditelo-7BS, suggesting its control by the B genome homoeoallele *Est-3*$_B$ in 7BS. $C^{3.15}$ is absent in nulli-7D/tetra-7A (16, Fig. 1) but is present in ditelo-7DS (17, Fig. 1), implying its control by the D genome homoeoallele in 7DS. The data of Figure 1 also suggest that the A genome homoeoallele of esterase C is not expressed in polyploid wheats.

The monomeric structure of esterase C is inferred from the absence of a hybrid isoenzyme between the two electrophoretically divergent homoeoallelic isoesterases in hexaploid wheats.

All linneons of hexaploid wheats are characterized by a common two-banded phenotype of esterase C. Only two mutant phenotypes, one lacking $C^{3.15}$ and the second one lacking $C^{3.4}$ have rarely come across.

Tetraploid wheats of the emmer group were characterized by the presence of $C^{3.4}$ controlled by the B genome homoeoallele. $C^{3.4}$ was lacking in 6 accessions of the wild emmer *T. dicoccoides* (out of 26 analyzed), 5 originating from Israel and one – from Syria. $C^{3.4}$ was also absent in the *timopheevii* group tetraploids characterized by the presence of a faint doublet of $C^{2.8}$ and $C^{3.0}$. The only

exception recorded was an accession of *T. araraticum* of Iranian origin which lacked the C-doublet and showed $C^{3.4}$ in common with emmers.

3.1.4 Esterase D

is specific to leaves and overlaps in electrophoretic mobility the coleoptile-specific esterase B. To distinguish electrophoretically esterases B and D, the coleoptile and leaf tissues of wheat seedlings should be analyzed separately.

Esterase D appears on the leaf enzymogram of hexaploid wheats as a broad band of two closely spaced isoesterases, $D^{4.35}$ and $D^{4.25}$, and a sharp band of isoesterase $D^{4.1}$. Enzymograms of nulli-6B/tetra-6D and ditelo-6BS strains of *T. aestivum* cv. 'Chinese Spring' (1 and 5, Fig. 3) reveal only the broad band and lack $D^{4.1}$ which is thus implied to be encoded by the B genome homoeoallele *Est-4*$_B$ located in 6BL. The nulli-6A/tetra-6D and ditelo-6Aα strains reveal isoesterases $D^{4.25}$ and $D^{4.1}$ but lack $D^{4.35}$ (3-4, Fig. 3), indicating that $D^{4.35}$ is encoded by the A genome homoeoallele *Est-4*$_A$ located in 6AL (formerly 6Aα). The nulli-6D/tetra-6B and ditelo-6DS (6Dα) strains produce $D^{4.35}$ and $D^{4.1}$ but lack $D^{4.25}$ (2 and 6, Fig. 3). This suggests that *Est-4*$_D$ encoding for $D^{4.25}$ is located in 6DL.

The same three D-isoesterases are common to all lineons of hexaploid wheats, variation being restricted to the silencing (appearing of null-mutants) of *Est-4*$_A$ and *Est-4*$_D$, whereas null-mutants of *Est-4*$_B$ were not encountered. The silencing of *Est-4*$_D$ was recorded only rarely, whereas the silencing of *Est-4*$_A$ was more common, especially among the accessions of *T. compactum* originating from Afghanistan and China and belonging to various botanical varieties. Null-mutants of esterase D were not encountered in *T. macha.*

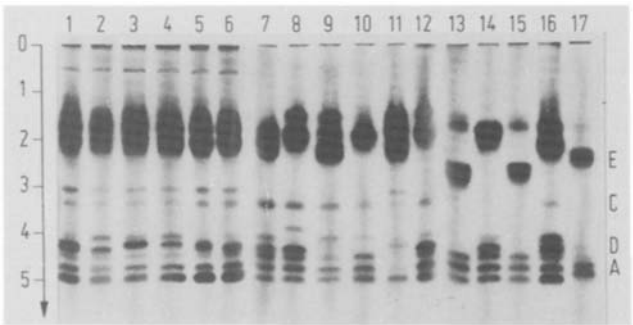

Fig. 3. Enzymograms of leaf esterases in polyploid wheats – chromosomal control and variation: 1-6 = nullisomics and ditelocentrics of *T. aestivum* cv. Chinese Spring: 1 = nulli-6B/tetra-6D; 2 = nulli-6D/tetra-6B; 3 = nulli-6A/tetra-6D; 4 = ditelo-6Aα; 5 = ditelo-6BS; 6 = ditelo-6Dα; 7-8 = *T. aethiopicum* var. *menelikii* (7) and var. *dominans* (8); 9-10 = *T. turgidum* var. *rubroalbum* from Spain (9) and var. *salomonis* from Bulgaria (10); 11 = *T. spelta* var. *rubrovelutinum* from Spain; 12 = *T. turgidum* var. *lusitanicum* from Turkey; 13 = *T. araraticum* from Armenia; 14 = *T. dicoccoides* from Israel; 15 = *T. araraticum* from Iraq; 16 = *T. dicoccon* from France; 17 = *T. araraticum* from Iran. The origin is at the top and the anode is at the bottom

Both *Est-4*$_A$ and *Est-4*$_D$ were found silenced only in two botanical varieties of *T. compactum*, var. *'rubriceps'* and var. *fetisowii*, which are both awned, with yellow seeds and redish spikes, but differ in the ear pubescense. All the 16 accessions of var. *'rubriceps'* (including the black-awned *'pseudo-rubriceps'*) analyzed lacked both $D^{4.35}$ and $D^{4.25}$, whereas some of the accessions of var. *'fetisowii'* lacked only $D^{4.35}$. The homologous couple of varieties of *T. aestivum* s.str., var. *erythroleucon* and var. *'turcicum'*, either revealed both $D^{4.35}$ and $D^{4.25}$ or lacked only one of them. The same is true for the awnless couple of varieties of *T. compactum, rufulum – crassiceps*, as well as for the couples *splendens-sericeum* and *erinaceum-echinoides* of *T. compactum*.

These data argue against the immediate phylogenetic link between *T. aestivum* var. *'turcicum'* and *T. compactum* var. *'rubriceps'*. The data indicate that the latter variety has evidently arisen monotopically from a biotype of *T. compactum* var. *'fetisowii'* already lacking both $D^{4.35}$ and $D^{4.25}$. This has not only led to the appearance of ear pubescence but has also accompanied by the mutation of $Est\text{-}1^{5.0}_{3D}$ to $Est\text{-}1^{5.2}_{3D}$, giving rise to the five-banded electrophoretic phenotype of esterase A described above.

Tetraploid wheats of the emmer group exhibit a common two-banded pattern of esterase D with isoesterases $D^{4.35}$ and $D^{4.1}$ present. The variation observed was restricted to the silencing of *Est-4*$_A$ with $D^{4.35}$ lacking (9-10, Fig. 3). Null-phenotypes of *Est-4*$_A$ were frequent among the accession of *T. turgidum* s.str., *T. durum*, and *T. polonicum*, but were rare in *T. dicoccoides, T. dicoccon* and *T. carthlicum*. No electrophoretic variants of *Est-4*$_A$ or silencing of *Est-4*$_B$ has been observed among emmers but *Est-4*$_B$ revealed an electrophoretic variant, $D^{3.9}$ (8, Fig. 3), found only in *T. aethiopicum*.

The leaf esterase enzymograms of *T. timopheevii* s.l. differ clearly from those of emmers (13-17, Fig. 3) in the absence of the two intense D-bands characteristic of emmers and reveal instead of them a faint band of different color shade at about 4.3. The enzymograms of leaf blades of older seedlings (7-12 days old) of *T. araraticum* show an intense band at 4.0 not detected in leaves of emmers and *T. timopheevii* s.str.

3.1.5 Esterase E

In addition to the four esterases specified above which comprise a group of fast-moving esterases with sharp bands, a group of closely-spaced slower-moving esterases is seen to form a broad and intensely stained banding on the esterase enzymograms (Figs. 1-3). Most accessions of *T.*

araraticum are characterized by the presence of a doublet of intensely stained leaf isoesterases at 2.6 and 2.8 not found in *T. turgidum* s.l. (Fig. 3, Jaaska 1971). *T. timopheevii* s.str. and two accessions of *T. araraticum* revealed (17, Fig. 3) a shift of the doublet towards lower electrophoretic mobility at about 2.3 and 2.5. The emmer wheats show a doublet with a still lower mobility at about 2.1 and 2.3 and intraspecific variation in its mobility.

A concomittant shift in the mobility of both bands in the same direction in intraspecific genetic variants suggests that they may reflect epigenetic molecular forms of the same esterase controlled by a single locus. We designate this esterase EST-E and its locus *Est-5*.

3.2 Diploids in Comparison with Polyploid Wheats

3.2.1 Esterase A

shows (Table 1) considerable variation among the wheat group diploids with up to five common and several rare electrophoretic variants (electromorphs).

These more extensive data confirm our previous report (Jaaska 1976) that the wild diploid wheat *T. boeoticum* exhibits intraspecific polymorphism of esterase A with three alloenzymes, $A^{4.6}$, $A^{4.5}$ and $A^{4.0}$, of which $A^{4.6}$ has become fixed in the cultivated *T. monococcum* s.str. and $A^{4.0}$ – in an another wild diploid, *T. urartu*. The most frequent alloenzyme in *T. boeoticum* was $A^{4.5}$ found in all accessions originating from Turkey (16), Lebanon (6) and Iran (6). $A^{4.6}$ characteristic of *T. monococcum* s.str. was observed in several accession of *T. boeoticum* from the Azerbaijan SSR (from 6 localities), Nakhitshevan ASSR (3) and Armenian SSR (6). $A^{4.0}$ characteristic of *T. urartu* has been found only in accessions of *T. boeoticum* collected in nature from local populations south-east of Yerevan (Armenian SSR).

Comparison of enzymograms in Figure 4 shows that $A^{4.5}$ of *T. boeoticum* exactly coincides with the slowest isoesterase of the esterase A triplet of polyploid wheats, whereas $A^{4.6}$ of *T. monococcum* s.str. and $A^{4.0}$ of *T. urartu* are clearly distinct and absent on the enzymograms of tetraploid wheats of both genetic groups. This result supports *T. boeoticum* carrying $A^{4.5}$ as the wheat A-genome donor and argues against *T. monococcum* s.str. and *T. urartu* in this role.

The alloesterases $A^{4.6}$ and $A^{4.0}$ are presumably mutational derivatives of the more ancestral $A^{4.5}$, since the latter is also characteristic of several wheatgrass species of

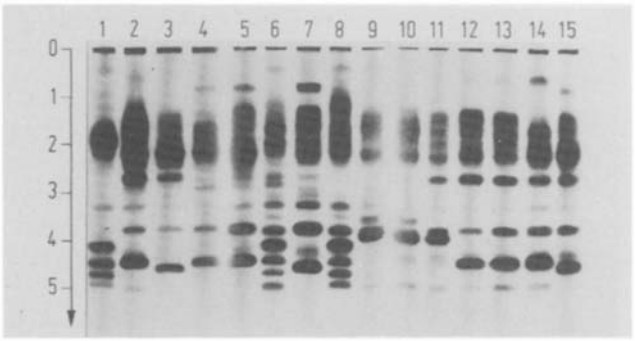

Fig. 4. Enzymograms of coleoptile esterases in diploid and tetraploid wheats: 1 = *T. dicoccoides* from Israel; 2-5 = *T. boeoticum* from Turkey (2) Armenia (3), Turkey (4), and from Azerbaijan (5); 6 = *T. timopheevii* from Georgia; 7 = *T. monococcum* var. *hornemannii* from the 'Zhanduri' population in Georgia; 8 = *T. dicoccon* from France; 9-10 = *T. urartu* from Armenia; 11-14 = *T. boeticum* from Armenia (11-13) and Iraq (14); 15 = *T. monococcum* var. *'laetissimum'* from Turkey. The origin is at the top and the anode is at the bottom

Table 1. Distribution of esterase A electrophoretic variants among diploids

Species	N	Number of accessions (N) with the variants							
		5.2	5.0	4.9	4.8	4.7	4.6	4.5	4.0
T. monococcum s.str.	23	0	0	0	0	0	23	0	0
T. boeoticum	84	0	0	0	0	0	20	63	3
T. urartu	19	0	0	0	0	0	0	0	19
Ae. tauschii	110	1	74	28	0	7	0	0	0
ssp. *tauschii*	51	1	16	27	0	7	0	0	0
ssp. *strangulata*	59	0	58	1	0	0	0	0	0
Ae. speltoides s.l.	49	0	49	0	0	1	0	0	0
Ae. bicornis	7	0	0	0	7	0	0	0	0
Ae. longissima s.str.	8	0	3	0	8	0	0	0	0
Ae. sharonensis	9	0	0	0	9	0	0	0	0
Ae. searsii	5	0	5	0	0	0	0	0	0
Ae. umbellulata	7	0	0	0	7	0	0	0	0
Ae. caudata	6	0	6	0	0	0	0	0	0
Ae. comosa s.l.	5	0	5	0	0	0	0	0	0
Ae. mutica	5	5	1	5	0	0	0	0	0

the genus *Agropyron* s.l. (unpublished data). *T. boeoticum* which shares all the three alloesterases may be considered as the most ancestral diploid wheat. *T. urartu* and *T. monococcum* s.str. have evidently diverged from different ancestral forms of *T. boeoticum* with $A^{4.0}$ and $A^{4.6}$, respectively, through the accumulation of gene mutations.

Diploid *Aegilops* (goatgrass) species are characterized by the presence of two frequent alloesterases, $A^{5.0}$ and $A^{4.8}$. Comparison of enzymograms in Figure 5 shows that the fastest isoesterase of the esterase A triplet of polyploid wheats corresponds to $A^{5.0}$ characteristic of *Ae. tauschii, Ae. speltoides* s.l., *Ae. searsii, Ae. caudata* and *Ae. comosa* s.l. The diploids *Ae. umbellulata, Ae. bicornis, Ae. sharonensis* and *Ae. longissima* s.str. share $A^{4.8}$ which has only rarely observed in some accessions of *T. araraticum*. Exceptionally, $A^{5.0}$ has been found as a rare alloenzyme segregating with $A^{4.8}$ in three accessions of *Ae. longissima*.

Diploid wheats and goatgrasses reveal a distinct genetic hiatus with respect to esterase A. $A^{5.0}$ and $A^{4.8}$ of goatgrasses were never encountered in diploid wheats, and, reversely, $A^{4.0}$, $A^{4.5}$ and $A^{4.6}$ were not found in diploid goatgrasses. The presence of $A^{5.0}$ in tetraploid wheats may thus be taken as a firm indication of the involvement of an *Aegilops* species in the origin of the wheat B-genome. *Ae. speltoides, Ae. longissima* and *Ae. searsii* are the suitable candidates, whereas *Ae. bicornis* and *Ae. sharonensis*, being fixed for $A^{4.8}$, are unsuitable.

No introgression of $A^{4.8}$ was observed in the accessions of *T. dicoccoides* originating from Israel where *Ae. longissima* also grows. $A^{4.8}$ found in some accessions of *T. araraticum* is presumably of mutational origin on the tetraploid level, since introgression from *Ae. longissima* s.l. is excluded because of distinctness of their distribution areas.

Ae. tauschii exhibits intraspecific polymorphism of esterase A with four electromorphs, $A^{5.0}$, $A^{4.9}$, $A^{4.7}$, and $A^{5.2}$. Almost all the variation observed was restricted to ssp. *tauschii*, whereas ssp. *strangulata* of different geographic origin (Transcaucasia, Iran, Turkmenia) was essentially monomorphic for $A^{5.0}$, $A^{4.9}$ being recorded in only one accession of the latter subspecies. In contrast, $A^{4.9}$ was the most common alloesterase for ssp. *tauschii* originating from Turkmenia and Afghanistan. However, $A^{5.0}$ was still most frequent among the accessions of ssp. *tauschii* from Transcaucasia where $A^{4.9}$ was rare.

Intraspecific and intrapopulational genetic polymorphism with three electromorphs which we label now $A^{4.9}$, $A^{5.2}$ and $A^{5.3}$ has previously been described for *Ae. mutica* (Jaaska 1974). $A^{4.9}$ and $A^{5.2}$ were most frequent in this species, whereas the three other variants $A^{5.3}$, $A^{5.1}$ and $A^{5.0}$, were recorded more rarely. In a reproductional sample, the three-banded heterozygotes of Est-$1^{4.9}$/Est-$1^{5.2}$ and the corresponding single-banded homozygotes were found to fit closely Hardy-Weinberg equilibrium, supporting the model of their genetic control by codominant alleles in a single locus encoding for a dimeric enzyme.

Null-mutants lacking the A-band were not observed in diploids, except a single seedling of *Ae. speltoides* among the hundred analyzed.

3.2.2 Esterase B

is represented in diploids by at least five electrophoretically distinct electromorphs (Table 2). Comparison of enzymograms in Figure 4 shows that $B^{3.9}$ of polyploid wheats controlled by the A genome homoeoallele closely coincides the B-band of *T. monococcum* s.l. (incl. *T. boeoticum*), whereas most accessions of *T. urartu* revealed a different alloesterase, $B^{3.6}$.

Comparison of enzymograms in Figure 5 shows that $B^{4.2}$ of polyploid wheats controlled by the B genome homoeoallele is also characteristic of several diploids, *Ae. speltoides* s.l., *Ae. comosa* s.l., *Ae. caudata* and *Ae. umbellulata. Ae. longissima, Ae. searsii* and *Ae. sharonensis* which belong to the Emarginata subsection of the section Sitopsis share a distinctly different alloesterase $B^{4.3}$ never observed in polyploid wheats. *Ae. bicornis*, a fourth species of the Emarginata subsection, has a distinct alloesterase, $B^{4.0}$, which is also characteristic of *Ae. tauschii* and exactly corresponds to that of hexaploid wheats controlled by the D genome homoeoallele.

Ae. speltoides is thus the only species of the section Sitopsis which is characterized by the esterase B electrophoretic variant corresponding to that of polyploid wheats controlled by the B genome homoeoallele. This

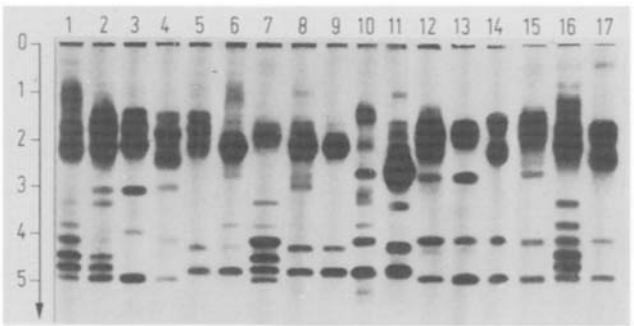

Fig. 5. Enzymograms of coleoptile esterases in diploid *Aegilops* species in comparison with those of polyploid wheats: 1 = *T. dicoccon* from France; 2 = *T. spelta* from Spain; 3 = *Ae. tauschii* ssp. *strangulata* from Azerbaijan; 4 = *Ae. caudata*; 5 = *Ae. sharonensis* from Israel; 6 = *Ae. longissima* from Israel; 7 = *T. dicoccoides* from Israel; 8 = *Ae. sharonensis*; 9 = *Ae. longissima*; 10 = *Ae. umbellulata* from Azerbaijan; 11 = *Ae. sharonensis* from Israel; 12 = *Ae. comosa* ssp. *comosa*; 13 = *Ae. comosa* ssp. *heldreichii*; 14 = *Ae. caudata* from Turkey; 15 = *Ae. speltoides*; 16 = *T. dicoccon* from France; 17 = *Ae. speltoides*. The origin is at the top and the anode is at the bottom

Table 2. Distribution of esterase B electrophoretic variants among diploids

Species	N	Number of accessions (N) with the variants					
		4.3	4.2	4.0	3.9	3.6	0
T. monococcum s.str.	23	0	0	0	23	0	0
T. boeoticum	84	0	0	0	84	0	0
T. urartu	19	0	0	0	3	16	0
Ae. tauschii	110	0	0	108	0	0	2
ssp. *tauschii*	51	0	0	50	0	0	1
ssp. *strangulata*	59	0	0	58	0	0	1
Ae. speltoides	49	0	41	0	2	0	24
Ae. bicornis	5	0	0	5	0	0	0
Ae. longissima s.str.	8	8	0	0	0	0	0
Ae. sharonensis	9	9	0	0	0	0	0
Ae. searsii	5	5	0	0	0	0	0
Ae. umbellulata	7	0	7	0	0	0	0
Ae caudata	6	0	6	0	0	0	0
Ae. comosa s.l.	5	0	5	0	0	0	0
Ae. mutica	5	0	0	0	0	0	5

result clearly favours *Ae. speltoides* s.l. (incl. *Ae. aucheri* auct.) as the wheat B genome donor and argues against any of the four species of the Emarginata subsection, *Ae. longissima, Ae. searsii, Ae. sharonensis* or *Ae. bicornis*, in the role of the full B genome donors. *T. urartu* is equally unsuitable for this role, since $B^{4.2}$ of polyploid wheats was not recorded in any accession of this species.

3.2.3 Esterase C

Diploid wheats *T. monococcum* s.str. and *T. boeoticum* are characterized by a common alloesterase $C^{2.8}$ which is also expressed in most accessions of *T. timopheevii* s.l., whereas *T. urartu* lacks esterase C (Table 3, Fig. 6). Null-mutants lacking esterase C were not encountered among the accessions of *T. monococcum* s.l. analyzed, whereas *T. urartu* seems to be fixed for a null-mutation of esterase C. Five accessions of *T. boeoticum*, originating from the Azerbaijan SSR (3), Turkey (1) and Lebanon (1), showed a faster variant, $C^{3.0}$, not observed in polyploid wheats.

The above data indicate a similarity between *T. urartu* and the emmer wheats in lacking $C^{2.8}$ and distinguish them from *T. timopheevii* s.l. and *T. monococcum* s.l. which share $C^{2.8}$. This result argues against *T. boeoticum* as the A genome donor of emmers and favours *T. urartu* in this role. *T. boeoticum*, on the contrary, shows homology with *T. araraticum* in sharing $C^{2.8}$.

Diploid *Aegilops* species revealed (Table 3) several electromorphs of esterase C and, in some species, null-mutants lacking esterase C in young seedlings. Most accessions of *Ae. tauschii* were characterized by the presence of $C^{3.15}$ which coincides with the D genome isoenzyme of hexaploid wheats (1-3, Fig. 5).

Aegilops speltoides s.l. displays extensive polymorphism of esterase C with at least four electromorphs, $C^{2.8}$, $C^{3.0}$, $C^{3.5}$ and $C^{3.8}$, the latter being the most frequent one. *Ae. longissima* and *Ae. sharonensis* share $C^{3.5}$ and $C^{3.15}$, of which only the latter was found in *Ae. searsii* and *Ae. bicornis*. Null-mutants lacking esterase C were also frequent in diploids of the Sitopsis section.

A local population of *Ae. mutica* south-east of Yerevan in the Armenian SSR showed intrapopulational polymorphism of esterase C with three electrophoretic variants, $C^{3.5}$, $C^{3.7}$ and $C^{4.0}$. The single-banded electrophoretic phenotypes of homozygotes and the doublets of heterozygotes were found to segregate in this population in a close agreement with Hardy-Weinberg equilibrium for three codominant alleles of a monomeric enzyme in a single locus.

A significant fact to point out is that $C^{3.4}$ character-

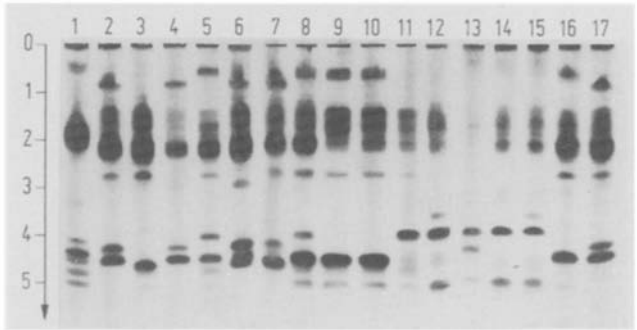

Fig. 6. Enzymograms of leaf esterases in diploid wheats: 1 = *T. dicoccoides* from Israel; 2-6 = *T. boeoticum* from Azerbaijan (2, 4, 6), Armenia (3), and Iraq (5); 7 = *T. monococcum* var. *'laetissimum'* from Turkey; 8-11 = *T. boeoticum* from Armenia; 12-15 = *T. urartu* from Armenia (12, 14, 15) and Lebanon (13); 16-17 = *T. boeoticum* from Iran (16) and Azerbaijan (17). The origin is at the top and the anode is at the bottom

Table 3. Distribution of esterase C electrophoretic variants among diploids

Species	N	Number of accessions (N) with the variants						
		3.8	3.5	3.4	3.15	3.0	2.8	0
T. monococcum s.str.	23	0	0	0	0	0	23	0
T. boeoticum	84	0	0	0	0	6	78	0
T. urartu	19	0	0	0	0	0	0	19
Ae. tauschii	110	0	0	4	99	0	0	7
ssp. *tauschii*	51	0	0	4	43	0	0	4
ssp. *strangulata*	59	0	0	0	56	0	0	3
Ae. speltoides	49	33	3	0	0	1	12	17
Ae. bicornis	7	0	0	0	6	0	0	1
Ae. longissima s.str.	8	0	0	0	5	0	0	5
Ae. sharonensis	9	0	4	1	3	0	0	4
Ae. searsii	5	0	0	0	5	0	0	0
Ae. umbellulata	7	0	0	0	0	0	7	0
Ae. caudata	6	0	0	0	0	0	0	6
Ae. comosa s.l.	5	0	5	0	0	0	0	0
Ae. mutica	5	5	5	0	0	0	0	0

istic of the B genome homoeoallele of emmer and dinkel wheats is not characteristic of any of the Sitopsis section diploids as well as of *T. urartu*. This may imply that a rare biotype of a Sitopsis species has contributed to the origin of the emmers' B genome. Alternately, it may be that $C^{3.4}$ of emmers has arisen mutationally on the tetraploid level in the course and as the result of differentiation of *T. dicoccoides* and *T. araraticum*. A possibility of such mutation is indicated by the occurrence of $C^{3.4}$ as a rare variant in one accession of *Ae. sharonensis* and four accessions of *Ae. tauschii* ssp. *tauschii*.

3.2.4 Esterase D,

a leaf-specific esterase, displays considerable inter- and intraspecific variation in diploids with at least six electrophoretic variants (Table 4, Figs. 6-7). Silencing of the esterase D gene (or its product) was a common feature in diploids.

The wild einkorn *T. boeoticum* showed two electrophoretic variants, $D^{4.25}$ and $D^{4.0}$, the first being the most frequent one and shared with the cultivated einkorn. *T. urartu* revealed two different variants, $D^{3.8}$ and $D^{4.35}$. The point of importance is that $D^{4.35}$ characteristic of the A genome homoeoallele $Est\text{-}4_{6A}$ of emmer and dinkel wheats was not observed in any accession of *T. monococcum* s.l. but was found for three accessions of *T. urartu* originating from Lebanon. This result suggests a possible contribution from *T. urartu* to the origin of the genome A of emmer and dinkel wheats and argues against the contemporary *T. boeoticum* as a sole donor of the full A genome. Alternately, such a biotype of *T. boeoticum* may still be found or a mutational change of $Est\text{-}4_A$ on the tetraploid level in *T. dicoccoides* may also be supposed.

Aegilops tauschii ssp. *strangulata* exhibits genetic variation with null-mutants and two electromorphs, $D^{4.25}$ and $D^{3.8}$, of which the first exactly coincides with the D genome-specific isoesterase of dinkel wheats (2-3, Fig. 7). *Ae. tauschii* ssp. *tauschii* is characterized by the presence of $D^{4.25}$ of reduced staining intensity on the leaf enzymograms (6, Fig. 7) in comparison with that of ssp. *strangulata* and of hexaploid wheats. $D^{3.8}$ was not encountered in the sample of ssp. *tauschii* analyzed and null-mutants were rare.

These data support a biotype of ssp. *strangulata* with $D^{4.25}$ as the contributor of the wheat D genome.

Aegilops speltoides displays extensive intraspecific and intrapopulational polymorphism of esterase D with several

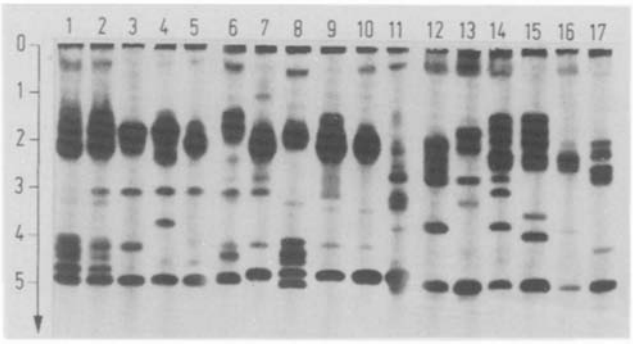

Fig. 7. Enzymograms of leaf esterases in diploid *Aegilops* species in comparison with those of polyploid wheats: 1 = *T. dicoccon* from France; 2 = *T. spelta* from Spain; 3-5 = *Ae. tauschii* ssp. *strangulata* from Iran (3-4) and Azerbaijan (5); 6 = *Ae. tauschii* ssp. *tauschii* from Afghanistan; 7 = *Ae. longissima* from Israel; 8 = *T. dicoccoides* from Israel; 9 = *Ae. sharonensis*; 10 = *Ae. longissima*; 11 = *Ae. umbellulata* from Azerbaijan; 12-17 = *Ae. speltoides* from Iraq (12-15) and Turkey (16-17). The origin is at the top and the anode is at the bottom

electromorphs (12-16, Fig. 7), three of which, $D^{4.25}$, $D^{3.8}$ and $D^{3.3}$, were observed most frequently. However, $D^{4.1}$ characteristic of the B genome in the emmer and dinkel wheats was inherent in *Ae. caudata*, but it was not encountered in the sample of *Ae. speltoides* analyzed, as well as in the remaining four diploids of the section Sitopsis. *Ae. longissima, Ae. searsii* and *Ae. sharonensis* share $D^{4.25}$ which is, as shows comparison of enzymograms 7-10 in Figure 7, distinctly different from the D-isoesterases of *T. dicoccoides*. No D-band was detectable on the leaf enzymograms of *Ae. bicornis* and *Ae. mutica*. The data in Table 6 reveal homologous variation of esterase D with common electromorphs and also frequent occurrence of null-mutants among diploids.

3.2.5 Esterase H

The coleoptile enzymograms of *Ae. tauschii* reveal, between the bands of esterases A and B, an esterase of independent variation pattern with two electromorphs labeled $H^{4.5}$ and $H^{4.7}$. The first variant was observed in ssp. *tauschii*, the second – in ssp. *strangulata*. Null-mutants of this esterase were frequent in ssp. *strangulata*, but rare – in ssp. *tauschii*. Esterase H was either lacking or of low activity in other diploids, as well as in polyploid wheats.

The other seedling esterases detectable electrophoretically will not be considered in this paper.

4 Discussion

4.1 On the Origin of Hexaploid Wheats

The overall electrophoretic similarity of seedling esterases in all linneons of hexaploid wheats supports a monophyletic origin of a primitive hexaploid wheat. Of specific importance in this respect is the fact that all linneons of hexaploid wheats share the same electrophoretic variants of esterases A, D and H, whereas *Ae. tauschii* exhibits intraspecific polymorphism of these esterases. In the case of recurrent origin of hexaploid wheats involving different biotypes of *Ae. tauschii* one would expect to observe in hexaploid wheats electrophoretic variants of these esterases.

The isoesterase data presented allow to specify the subspecies and biotype of *Ae. tauschii* which has contributed to the origin of the primitive hexaploid wheat. The subspecies *tauschii* is characterized by a high frequency of alloesterases $A^{4.9}$, $A^{4.7}$, $H^{4.5}$ and $D^{4.25}$ of reduced activity which makes it unsuitable as the wheat D genome donor. Biotypes of ssp. *strangulata* with the alloesterases $D^{3.8}$ and $H^{4.7}$ are equally unsuitable. A biotype of ssp. *strangulata* with the esterases $A^{5.0}$, $B^{4.0}$, $C^{3.15}$, $D^{4.25}$ and H^{0} fits best the wheat D genome donor. Subspecies *strangulata* was also favoured as the D genome donor on the basis of α-amylase isoelectrofocusing (Nishikawa 1973) and of seed protein electrophoretic (Konarev et al. 1976) data.

The isoesterase data, however, fail to indicate which linneon of emmers has contributed to the origin of the primitive hexaploid wheat or which of the contemporary hexaploids is the most primitive.

It must be emphasized that the concept of monophyletic origin of the primitive hexaploid wheat in Transcaucasia where ssp. *strangulata* is spread in no case does not contradict to the recurrent polytopic origin of contemporary linneons of hexaploid wheats through the accumulation of mutations and sporadical introgression from tetraploid wheats which is experimentally well substantiated (Dorofeev 1971; Gandilian 1972).

4.2 On the Origin of Tetraploid Wheats

The essential identity of electrophoretic patterns of esterases A and B in both genetic groups of tetraploid wheats comprising *T. turgidum* s.l. and *T. timopheevii* s.l. may be taken as an evidence in favour of their origin from a common primitive tetraploid precursor. The electrophoretic phenotypes of esterases A and B in tetraploid wheats reveal codominant expression of alloesterases characteristic of contemporary diploids *T. boeoticum* and *Ae. speltoides*. *T. monococcum* s.str. and *T. urartu* are fixed for electrophoretically divergent variants of esterase A and may thus be rejected as donors of the full wheat A genome. Similarly, *Ae. longissima* s.str., *Ae. searsii, Ae. sharonensis* and *Ae. bicornis* are unsuitable as the wheat B genome donors, since their esterase $B^{4.3}$ is electrophoretically distinctly different from the B genome isoesterase $B^{4.2}$ of polyploid wheats.

Esterases C, D and E, however, show clear genetic differences between the emmer and timopheevii groups of tetraploid wheats. These data also cast doubt on the idea that contemporary tetraploid wheats are simple evolutionary derivatives of a primitive amphidiploid which combines the genomes of *T. boeoticum* and *Ae. speltoides*. Indeed, isoesterases $D^{4.35}$ and $D^{4.1}$ of emmer and common wheats which are controlled by their A and B genomes, respectively, were not observed in the analyzed sample of *T. boeoticum* and in any of the *Sitopsis* diploids but were found in *T. urartu* and *Ae. caudata*, respectively.

There is a general agreement among triticologists about the origin of the genome A of polyploid wheats (reviewed by Riley 1965; Kimber 1974). A wild diploid wheat, *T. boeoticum* or *T. urartu*, is widely accepted as the A ge-

Table 4. Distribution of esterase D electrophoretic variants among diploids

Species	N	Number of accessions (N) with the variants						
		4.35	4.25	4.1	4.0	3.8	3.3	0
T. monococcum s.str.	23	0	23	0	0	0	0	0
T. boeoticum	84	0	65	0	6	0	0	15
T. urartu	19	3	0	0	0	14	0	2
Ae. tauschii	110	0	61	0	0	32	0	17
ssp. *tauschii*	51	0	47	0	0	0	0	4
ssp. *stangulata*	59	0	14	0	0	32	0	13
Ae. speltoides	49	0	4	0	0	6	32	36
Ae. bicornis	4	0	0	0	0	0	0	7
Ae. longissima s.str.	8	0	7	0	0	0	0	1
Ae. sharonensis	6	0	3	0	0	0	0	3
Ae. searsii	5	0	5	0	0	0	0	0
Ae. umbellulata	7	0	0	1	0	6	0	0
Ae. caudata	6	0	0	6	0	0	0	0
Ae. comosa s.l.	5	5	0	0	0	0	0	0
Ae. mutica	5	0	0	0	0	0	0	5

nome donor of polyploid wheats. On the basis of immunochemical properties of a seed protein, Konarev (1975) has proposed that the A genome of emmers is derived from an ancestor of *T. urartu*, whereas the A genome of *T. timopheevii* s.l. was contributed by *T. monococcum* s.l.

Our previous studies on acid phosphatase (Jaaska 1974, 1976) and NAD-dependent alcohol dehydrogenase (Jaaska 1976a) isoenzymes as well as the esterase D data reported in the present paper have discovered the existence of distinct genetic differences between the A genomes of contemporary *T. boeoticum, T. urartu* and polyploid wheats. It has been found (Jaaska 1974, 1976) that the isophosphatase doublet of *T. monococcum* s.l. differs electrophoretically from the isophosphatases of polyploid wheats and *T. urartu. T. turgidum* s.l., *T. timopheevii* s.l. and *T. urartu* were found to share an electrophoretically coinciding isophosphatase doublet which is controlled by the B genome homoeoallele in emmers and by the A genome in the timopheevii group. The acid phosphatase electrophoretic pattern of *T. timopheevii* s.l. was shown to comprise the sum of isophosphatases characteristic of *T. urartu* and frequent in *Ae. speltoides*, whereas *Ae. searsii* (unpublished data), *Ae. longissima, Ae. sharonensis* and *Ae. bicornis* all share an isophosphatase doublet distinctly different from those of polyploid wheats.

To explain the acid phosphatase and esterase A isoenzyme data it has been suggested (Jaaska 1976) that both *T. boeoticum* and *T. urartu* might have contributed to the origin of both groups of tetraploid wheats. One of the possibilities to be considered may be the involvement of a male-sterile hybrid between *T. urartu* and *T. boeoticum* as a female parent which was pollinated by *Ae. speltoides* or its close relative to produce a primitive allotetraploid from which *T. araraticum* and *T. dicoccoides* later diverged, presumably through additional hybridization events and accumulation of mutations. These speciation events may have caused some rearrangements of chromosomes of the parental species between the two genomes of tetraploid wheats which may thus not fully correspond to any genome of contemporary diploids.

A possibility has also been discussed (Jaaska 1976a) that the observed electrophoretic differences in the acid phosphatase and NAD-dependent alcohol dehydrogase (ADH) isoenzymes of *T. boeoticum* and of the A genome of *T. turgidum* s.l. may have arisen mutationally on the tetraploid level during the evolutionary divergence of *T. dicoccoides* from *T. araraticum* which has led to the fixation of new alleles in the two loci in the emmer group. However, it is not yet excluded that a biotype of *T. boeoticum* with the acid phosphatase, NAD-ADH and esterase D isoenzymes corresponding electrophoretically to those of the emmers' A genome may still be found by further electrophoretic studies of this species.

The origin of the wheat B genome is continuing to be the subject of controvercy and extensive discussions, as reviewed by Kimber (1974), Riley (1965) and others.

The most recent proposal for the wheat B genome donor is *Ae. searsii* – a recently discovered new diploid species closely related to *Ae. longissima* but differentiated from it morphologically and ecologically (Feldman and Kislev 1977; Feldman 1978; Feldman et al. 1979). The isoesterase data reported in this paper as well as our preliminary unpublished data on other enzymes confirm close genetic affinities between *Ae. searsii* and *Ae. longissima* but they fail supporting neither of them as the wheat B genome donor.

Our previous electrophoretic studies of acid phosphatase (Jaaska 1974, 1976), aspartate aminotransferase (Jaaska 1976b) and NADP-ADH (Jaaska 1978) have favoured *Ae. speltoides* as the wheat B genome donor and have evidenced against the diploids of the Emarginata subsection of the section Sitopsis as well as against *T. urartu* in this role. *Aegilops speltoides* was shown (Jaaska 1978) to be the sole diploid possessing the NADP-ADH isoenzyme corresponding electrophoretically to that controlled by the long arm[1] of the chromosome 5B in polyploid wheats.

However, the acid phosphatase, esterase C and D isoenzymes controlled by the B genome homoeoalleles in the emmer wheats were either rare or not encountered in the analyzed sample of *Ae. speltoides*. This indicates that the alleles of some enzymes fitting the emmers' B genome are rare in the contemporary *Ae. speltoides* or may even be lost.

Aegilops speltoides and *Ae. mutica* are the only two self-incompatible cross-pollinating species in the genus *Aegilops*. Cross-fertilization is generally recognized (Stebbins 1957; Fryxell 1957; Mac Key 1970; Lewis 1973) as a primitive, ancestral breeding system from which selfers recurrently diverge through mutations in the genetic system controlling self-incompatibility. On this general theoretical grounds, *Ae. speltoides* may be considered as the most ancestral species in the section Sitopsis. Contemporary autogamous species of the Emarginata subsection of the section Sitopsis, *Ae. longissima, Ae. searsii* and *Ae. bicornis*, have presumably diverged from different ancestral forms of *Ae. speltoides* in the Palestine region through the acquisition of self-compatibility and fixation of mutations.

It is also recognized (Grant 1956) that the origin of allopolyploidy is more frequently connected with self-compatible precursors rather than with self-incompatible outbreeders, whereas the hybridization involving cross-fertilizing species usually leads to introgression but not to polyploidy. On this basis it may be assumed that ancestral self-compatible biotypes of *Ae. speltoides* have contributed to the origin of the wheat B genome. These biotypes, however, were evidently different from those from which contemporary autogamous species of the section Sitopsis have emerged.

The observed extensive intraspecific genetic variation in *Ae. speltoides* at several esterase loci as well as at the acid phosphatase locus indicates that this species is a rich storehouse of allelic resources which may be of potential value in the wheat breeding programs. Genetic variation in natural populations of *Ae. speltoides* should be studied more closely to establish the extent of this variation and to search for self-fertile biotypes which fit genetically the requirements for the wheat B genome donor.

1 Erroneously specified as a short arm in our original paper, l.c.

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Received August 25, 1979
Accepted October 12, 1979
Communicated by J. Mac Key

Dr. V. Jaaska
Laboratory of Biochemistry
Institute of Zoology and Botany
21 Vanemuise Street
202400 Tartu, Estonian SSR (USSR)