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The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat

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Abstract Polymorphism in the lengths of restriction fragments at 53 single-copy loci, the rRNA locus *Nor3*, and the high-molecular-weight glutenin locus *Glu1* was investigated in the D genome of hexaploid *Triticum aestivum* and that of *Aegilops tauschii*, the source of the *T. aestivum* D genome. The distribution of genetic variation in *Ae. tauschii* suggests gene flow between *Ae. tauschii* ssp. *strangulata* and ssp. *tauschii* in Iran but less in Transcaucasia. The "strangulata" genepool is wider than it appears on the basis of morphology and includes ssp. *strangulata* in Transcaucasia and southeastern (SE) Caspian Iran and ssp. *tauschii* in northcentral Iran and southwestern (SW) Caspian Iran. In the latter region, *Ae. tauschii* morphological varieties 'meyeri' and 'typica' are equidistant to ssp. *strangulata* in Transcaucasia, and both belong to the "strangulata" genepool. A model of the evolution of *Ae. tauschii* is presented. On the geographic region basis, the D genomes of all investigated forms of *T. aestivum* are most closely related to the "strangulata" genepool in Transcaucasia, Armenia in particular, and SW Caspian Iran. It is suggested that the principal area of the origin of *T. aestivum* is Armenia, but the SW coastal area of the Caspian Sea and a corridor between the two areas may have played a role as well. Little genetic differentiation was found among the D genomes of all investigated free-threshing and hulled forms of *T. aestivum*, and all appear to share a single D-genome genepool, in spite of the fact that several *Ae. tauschii* parents were involved in the evolution of *T. aestivum*.

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Introduction

Triticum aestivum L. (genomes AABBDD) comprises a number of morphological forms (subspecies) differing from each other by a single or a few major genes (Mac Key 1966). In ssp. *spelta* (L.) Thell. (spelt wheat), ssp. *macha* (Dekapr. & Menabde) Mac Key, ssp. *vavilovii* (Jakubz.) A. Love, and ssp. *yunnanense* King glumes adhere tightly to the kernels at maturity and, consequently, they are hulled. In ssp. *aestivum* (bread wheat), ssp. *compactum* (Host) Thell. (club wheat), and ssp. *sphearococcum* (Perc.) Mac Key, *T. petropavlovskyi* Udach. & Migush. (rice wheat), and ssp. *tibetanum* Shao (Tibetan wheat) kernels are free of glumes at maturity and, consequently, they are free-threshing.

There is extensive genetic evidence that *T. aestivum* originated by hybridization of tetraploid *T. turgidum* L. (genomes AABB) with diploid *Aegilops tauschii* Coss. (genomes DD) (Kihara 1944; McFadden and Sears 1946). McFadden and Sears (1946) concluded that the hulled spelt is ancestral to the free-threshing forms of hexaploid wheat. Spelt was first recognized in Europe. Several early reports of spelt cultivation in Asia (Jakubziner 1958) fell into obscurity until Kuckuck's rediscovery of spelt in Iran (Kuckuck and Schiemann 1957). The existence of spelt in Asia reinforced McFadden's and Sears' (1946) hypothesis (Kuckuck and Schiemann 1957; Kuckuck 1959; Andrews 1964). However, the occurrence of free-threshing hexaploid wheat before spelt in archeological sites nevertheless raises valid concern about the ancestral position of spelt to free-threshing wheat (for recent review see Nesbitt and Samuel 1996). An alternative scenario to that proposed by

McFadden and Sears is that spelt was derived by introgressive hybridization between a free-threshing hexaploid wheat and the hulled *T. turgidum* ssp. *dicoccon* (Schrank.) Thell. (Mac Key 1966; Tsunewaki 1968; Liu and Tsunewaki 1991). Jaaska (1978) concluded from allozyme variation in the A genome that the European spelt originated independently of the Asian spelt. Accordingly, the European spelt could have originated by introgressive hybridization of *T. aestivum* with *T. turgidum* ssp. *dicoccon* whereas the Asian spelt could represent the ancestral form of hexaploid wheat. It is also possible that at least some of the hulled wheats originated from different amphiploids as speculated, for example by Dekaprelevich (1961) and Swaminathan (1966).

Because of the possibility of hybridization between tetraploid and hexaploid wheat, the A and B genomes are less reliable sources of information on the relationships among the hexaploid forms of wheat than is the D genome. Because the D genome does not recombine in such crosses, its genetic relationships with the genepool of *Aegilops tauschii* can potentially provide valuable information on the relationships among the various forms of hexaploid wheat and on the various scenarios of the origin of spelt and other forms of *T. aestivum*. *Aegilops tauschii* encompasses four morphological varieties, of which three ('typica', 'anathera', and 'meyeri') are grouped into ssp. *tauschii*, whereas ssp. *strangulata* (Eig) Tzvelev is monotypic. Subspecies *tauschii* is distributed from eastern Turkey to China and Pakistan, whereas ssp. *strangulata* occurs in two disjoined regions, southeastern (SE) Caspian Iran and Transcaucasia (Kihara et al. 1965; Yen et al. 1984; van Slagern 1994; Jaaska 1995).

Variation in isozymes was used to identify the *Ae. tauschii* subspecies that contributed the D genome to *T. aestivum*. The spectrum of alleles at the α-amylase loci in *T. aestivum* corresponds better to the allele spectrum in ssp. *strangulata* than to that in ssp. *tauschii* (Nishikawa 1974; Nishikawa et al. 1980). Jaaska (1978, 1981) identified fixed polymorphisms at the aspartate aminotransferase and aromatic alcohol dehydrogenase loci between *Ae. tauschii* subspecies, and in both cases the ssp. *strangulata* isozymes had the same mobility as those found in the *T. aestivum* D genome. He also pointed out that the profile of esterase isozymes characterizing the D genome of *T. aestivum* fits better that in ssp. *strangulata* than that in ssp. *tauschii* (Jaaska 1980).

Since ssp. *strangulata* is distributed in two regions, there are two choices as to the birthplace of *T. aestivum*. Jaaska (1980) considered Transcaucasia to be the center of the distribution of ssp. *strangulata* and hence assigned the origin of *T. aestivum* there. Nishikawa at al. (1980) found that the α-amylase isozyme profile present in *T. aestivum* is more prevalent in SE Caspian Iran than in Transcaucasia and therefore suggested that the most likely birthplace of *T. aestivum* is SE Caspian Iran. Tsunewaki (1966) considered *Ae. tauschii* in southwestern (SW) Caspian Iran and nearby mountainous Azerbaijan as the source of the *T. aestivum* D genome because of the distribution of the waxy-bloom allele. Nakai's (1979) studies of esterase isozymes provided additional support for SW Caspian Iran and Transcaucasia as the putative places of origin of *T. aestivum*.

Restriction fragment length polymorphism (RFLP) at 20 polymorphic, single or low-copy-number loci has been used in the investigation of relationships among *Ae. tauschii* accessions (Lubbers et al. 1991). The greatest amount of variation was found in *Ae. tauschii* accessions collected in Iran and western Transcaucasia. Variety 'meyeri', although formally placed in ssp. *tauschii*, was found to be genetically closer to ssp. *strangulata* than to ssp. *tauschii*. A similar finding was made by Tsunewaki et al. (1991) on the basis of RFLP analysis of several accessions of *Ae. tauschii*. Lubbers et al. (1991) concluded that var 'meyeri' actually belongs to ssp. *strangulata*.

Several studies on the relationships between wheat and *Ae. tauschii* employing variation in DNA have been reported. Tsunewaki et al. (1991) hybridized 46 genomic clones with Southern blots of DNAs of several *Ae. tauschii* accessions and *T. aestivum* cv 'Chinese Spring' (ssp. *aestivum*). An accession of subspecies *strangulata* and an accession of var 'meyeri' appeared to be equidistant to the D genome of 'Chinese Spring', whereas the accessions of var 'typica' were more distant. Lagudah et al. (1991) pointed out that the rRNA gene non-transcribed spacer (NTS), high-molecular weight glutenin subunits and esterase isozymes of ssp. *strangulata* match those of the *T. aestivum* D genome better than those of ssp. *tauschii*.

RFLP at 53 single-copy loci was employed here in an investigation of genetic relationships among accessions of *Ae. tauschii* and the D genome of *T. aestivum*. Single-copy loci usually have simple restriction fragment profiles, and their use minimizes the possibility of allocation of polymorphic restriction fragments to an erroneous genome in hexaploid wheat. In addition to these loci, variation at the high-molecular-weight glutenin locus (*Glu1*) and the highly polymorphic 18S-26S rRNA gene NTS was employed in this study.

Materials and methods

Plant materials

RFLP was investigated in 172 accessions of *Ae. tauschii*, 178 accessions of *T. aestivum* ssp. *aestivum* and ssp. *compactum*, including Chinese endemic wheats (Table 1), 64 accessions of spelt, 10 accessions of *T. aestivum* ssp. *macha*, and 2 accessions of *T. aestivum* ssp. *vavilovii* (Table 1). The spelt accessions consisted of 52 accessions of European spelt and 12 accessions of Asian spelt from four geographic regions (Table 1). *Triticum aestivum* accessions were grouped according to subspecies and the geographic region of origin, numbered 1–6 (Table 1). *Aegilops tauschii* accessions were

Table 1 Accessions and their geographic origin

Species	Subspecies	Geographic origin	Group no.	Accession[a]
T. aestivum	*aestivum*	Western region	1	Cheyenne, Anza, Canthatch, Dirkwin, Inia 66R, Klasic, Lerma Rojo, Marquis, Newton, Ramona, Red Chief, Serra, Siete Ceros, Turkey, Yamhill, Yecora Rojo, 4 accessions of var *carthlicoides*
	aestivum	Eastern region (China)	2	Chinese Spring, 4 landraces from Guizhou, 5 from Hebei, 6 from Hubei, 11 from Hunan, 5 from Jiangsu, 42 from Shaanxi, 5 from Shandong, 5 from Shanghai, 6 from Shanxi, 29 from Sichuan, 6 from Tibet, 5 from Xinjiang, 8 from Yunnan, 5 from Zheijiang
	yunnanense	Yunnan (China)	2	AS331, AS334, AS335, AS336, AS337, AS338, AS340, AS341
	tibetanum	Tibet (China)	2	AS329,AS330,AS907,AS908,AS1025,AS1027
	petropavlovskyi	Xinjiang (China)	2	AS358, AS359, AS362, AS363, AS2338, 95-939, 95-941, Akesu rice wheat, Yutian rice wheat
	compactum	USA	1	Big Club 60
	macha	Georgia	3	VIR58652, VIR28181, G1497, G531, G532, G533, G534, G535, G1260, G1488,
	spelta	Afghanistan	4	PI367199, PI367201, PI367202, PI367203
	spelta	Iran	4	Kuckuck 405a, 77d, P543, 407a, 417a,
	spelta	Tadjikistan	4	VIRk-52442, VIR 56569,
	spelta	Azerbaijan	4	VIR45366,
	spelta	Europe	5	5 from Spain, 2 from Yugoslavia, 5 from England, 5 from Hungary, 1 from Poland, 5 from Bulgaria, 5 from Romania, 2 from Austria, 5 from Belgium, 5 from Switzerland, 5 from Germany, 2 from Italy, 2 from Macadonia, 3 origin unknown
	vavilovii	Unknown	6	G5085, G530
Ae. tauschii	*tauschii*	Turkey	7	KU2131, KU2132, KU2133, KU2134, KU2136, KU2138, KU2140, KU2141
	tauschii	Western Iran (Mahabad region)	8	KU2113, KU2114, KU2115
	tauschii	Georgia	9	KU2826, KU2827[b], KU2828[b], KU2832, KU2829 A, KU2829B, KU2835B
	tauschii	Armenia	9	AL8/78-1, -2, -3, AL9/78-1, -2, -3, -4, -5, -6, -7, -8, -9, KU2808, KU2810, KU2811[b], KU2814, KU2816, KU2821, KU2823, KU2824, KU2822-A,-B
	strangulata	Armenia	10	AL7/80-1, -3, -5, -9, AL11/80-1, -2, -3, -4
	tauschii	Azerbaijan-Nakhichewan	9	Al10/80-1, -2, -3, -4, AL46/77-3, -4, KU2111[b], KU2112[b], KU2117[b], KU2118[b], KU2120, KU2121, KU2122, KU2123[b], KU2142, KU2143, KU2144, KU2145, KU2148, KU2151, KU2804[b], KU2806[b], KU2808[b], KU2144, KU2144, KU2144, KU2144, KU2144
	strangulata	Azerbaijan, Nakhichevan	10	K1574/72-1, 9, Al10/80-3, -4
	tauschii	Southwestern Caspian (Chalus to Astara)	11	KU2098, KU2100, KU2101, KU2102, KU2103, KU2104, KU2105, KU2106, KU2108, KU2110, KU2158, KU2159, KU2160, KU2061, KU20-10
	tauschii	North-central Iran (Teheran region)	12	KU2068, KU2069, KU2071, KU2086, KU2154, KU2155, KU2156, KU20-7, KU20-8
	tauschii	Southeastern Caspian Iran (Behshahar-Gorgan region)	13	KU2124, KU2125, KU2126, KU2471, KU2082, KU2083
	strangulata	Southeastern Caspian Iran (Behshahar-Gorgan region)	14	AL370/77-2, RL5288-1, -2, KU,2080, KU2087, KU2088, KU2091, KU2095, KU2124, KU2115, KU2110, KU21111, KU2074, KU2076, KU2078, KU2079, KU2088, KU2090, KU2091, KU2092, KU2093, KU2095, KU20-9
	tauschii	Turkmenistan	15	AL20/79-2, -4, -6, -9, AL25/79-1, -3, -4, AL7/79-1, -2, -3, -7
	tauschii	Northwestern Afghanistan	16	KU2022, KU2032, KU2035, KU2039, KU2044, KU2047, KU2048, KU2050, KU2056, KU2058, KU2059
	tauschii	Eastern Afghanistan	16	KU2063, KU2066, KU20-4
	tauschii	China	17	82-Ae5, 82-Ae42, 82-Ae46, AS71, AS72, AS75, AS76, AS77, AS78, AS79, AS80, AS81, AS82

[a] Accessions designated by a number preceded by G were collected by L.B. Johnson and supplied by G. Waines (University of California Riverside, Calif); those preceded by KU were collected by H. Kihara, H. Yamashita, and M. Tanaka (Kyoto University, Kyoto, Japan) and were supplied by B.S. Gill (Kansas State University), those preceded by AL or K were collected by V. Jaaska (Estonian University, Tartu, Estonia). Accessions preceded by Ae were supplied by Dr. Y.-S. Dong (Chinese Academy of Agricultural Sciences, Beijing), those preceded by RL by E. Kerber (Agriculture Canada, Winnipeg, Canada), those preceded by AS and other Chinese accessions by Prof. C. Yen (the Triticeae Research Institute, Sichuan Agricultural University, China). Accessions originally collected by H. Kuckuck were supplied by E. R. Sears (University of Missouri, Columbia). Accessions preceded by PI and the European speltas were supplied by H. Bockelman, USDA Small Grains Colection, Aberdeen, Idaho

[b] Accessions received as ssp. *tauschii* but reclassified into ssp. *strangulata* on the basis of data in Table 4

grouped by subspecies and the geographic region of origin into 11 groups (Fig. 1), numbered 7–17 (Table 1). All *Ae. tauschii* accessions were grown to maturity in the field, and the spike morphology was compared with the allocation of the accessions into subspecies and varieties by collectors.

RFLP

Nuclear DNAs were isolated from leaves (Dvorak et al. 1988) and digested with *Dra*I or *Xba*I. Southern blotting, DNA hybridization, and probes have been described earlier (Dvorak et al. 1998a). Only clones that hybridized with one or a few cosegregating fragments mapped in the DNAs of a *T. monococcum* mapping population (Dubcovsky et al. 1996) were employed in this study. The position of each locus in the wheat D genome was confirmed by synteny mapping using *T. aestivum* nullisomic-tetrasomic stocks (Sears 1966) and *T. aestivum* disomic substitution lines harboring single chromosome pairs of *Lophopyrum elongatum* substituted individually for wheat homeologous chromosome pairs (Dvorak 1980; Dvorak and Chen 1984).

Variation at 55 loci was investigated. Of these loci 51 were identical to those reported in a previous paper (Dvorak et al. 1998a). The following are additional single-copy loci not previously reported by Dvorak et al. (1998a): *Xmwg503-5D*, *Xpsr547-1D*, and *Xbcd98-7D*. In addition, the rRNA locus *Nor3* (chromosome 5D) was investigated. The 55 loci covered all seven chromosomes and both arms of each chromosome. The positions of these loci on the diploid wheat genetic map are shown in Dvorak et al. (1998a).

In *Ae. tauschii*, RFLP at 53 loci was investigated in *Dra*I-digested DNAs. For the *Nor3* and *XGlu1* loci, *Taq*I and *Xba*I-digests were employed, respectively. For 15 of the 53 single-copy loci, RFLP was also investigated in *Xba*I-digested DNAs. Thus, a total of 70 enzyme × probe combinations were used for *Ae. tauschii*.

RFLP at the same 70 enzyme × probe combinations was also investigated in all accessions of *T. aestivum* ssp. *macha* and *vavilovii*, a limited sample of European and Asian spelt, and reference cv 'Chinese Spring'. Since some loci were monomorphic in *Ae. tauschii* or not informative in wheat, a subpopulation of 27 most informative loci was selected for the investigation of genetic relationships between *Ae. tauschii* and *T. aestivum*, employing all accessions listed in Table 1. RFLP at 21 loci was investigated with *Dra*I, 2 loci with *Xba*I, 2 loci with both *Dra*I and *Xba*I, 1 locus with *Taq*I, 1 one locus with *Eco*RV. Thus, a total of 29 enzyme × probe combinations was used.

Data analyses

The lengths of restriction fragments were used as the basis of estimating polymorphism. An allelic designation was assigned to each restriction fragment length pattern, and the frequency of each allele was computed. The allelic frequencies were used to compute gene diversity h at a locus (Nei 1987). The h values were used as variables in the estimation of average H over all loci (Nei 1973). Allelic frequencies, H, and the variance of H were computed with the POPGENE program (Yeh et al. 1997). Differences between values of H in different populations were tested by the paired t-test. The magnitude of divergence between populations was computed as Nei's genetic distance D (Nei 1978). The D values were computed with the Genetic Distance Analysis (GDA) computer program (Lewis and Zaykin 1997). Phenograms using the neighbor-joining method were constructed with the GDA program.

Results

Gene diversity

Of 70 enzyme × probe combinations, 49 (70%) detected polymorphism among the 172 accessions of *Ae. tauschii*. Of 51 *Dra*I digests, 27% were monomorphic and of 19 *Xba*I digests 37% were monomorphic. Of 15 loci investigated with both endonucleases, 20% were monomorphic in both digests, while only 10% were expected on the basis of single digests ($0.27 \times 0.37 \times 100$).

The *Nor3* locus was the most variable. A total of 15 NTS patterns were found in *Ae. tauschii*: 14 are shown in Fig. 2; pattern r is not shown. Patterns h and v (Fig. 2) were found in accessions from Uzbekistan and Azerbaijan that were not included in this study.

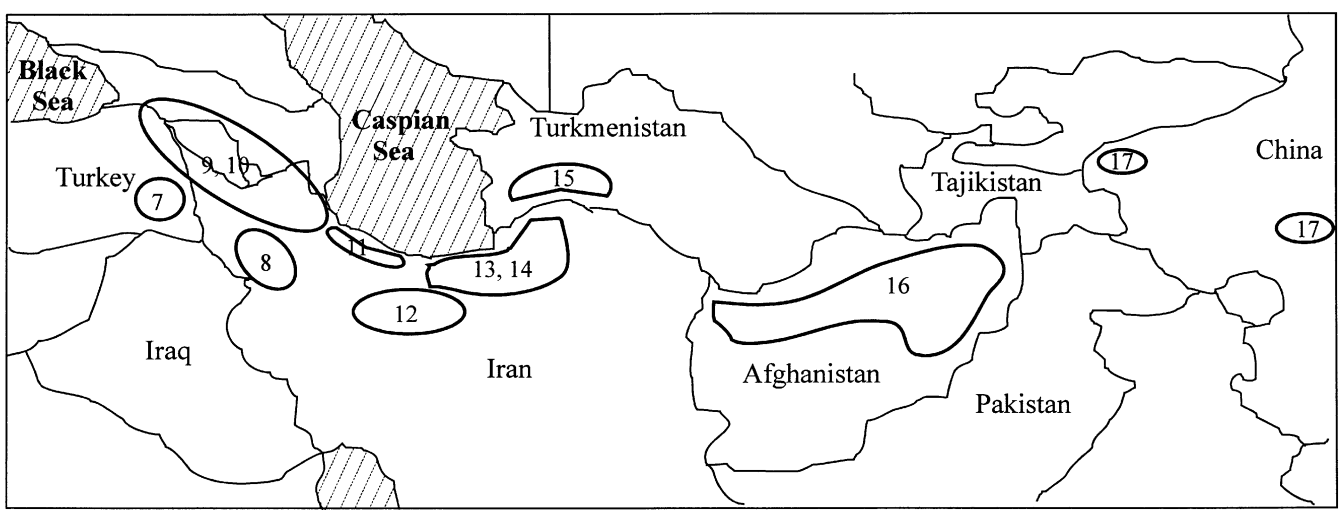

Fig. 1 Approximate geographic location of groups of *Ae. tauschii* accessions. *Groups 9* and *10* and *13* and *14* are sympatric ssp. *tauschii* and ssp. *strangulata*. The remaining populations are ssp. *tauschii*

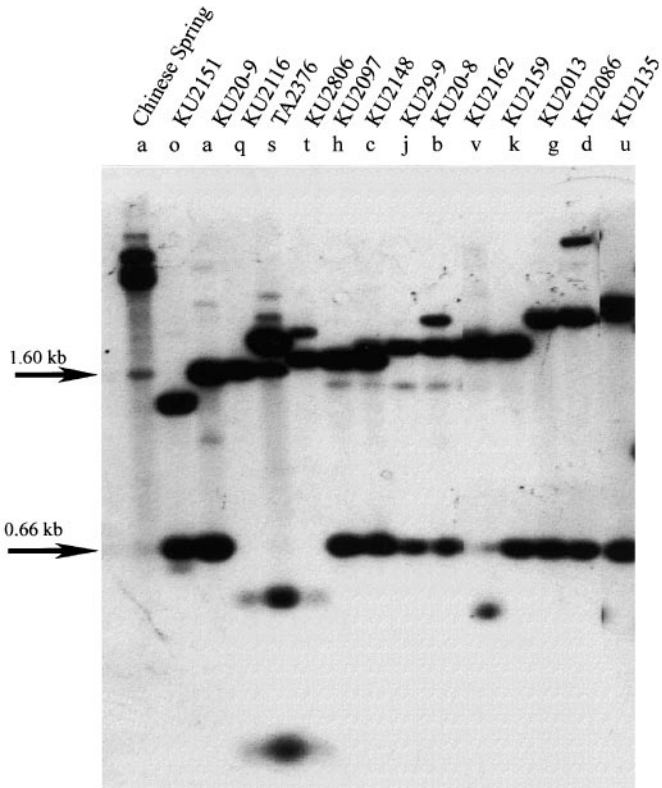

Fig. 2 An autoradiogram of a Southern blot of DNAs of indicated *Ae. tauschii* accessions and 'Chinese Spring' digested with the *Taq*I restriction endonuclease and hybridized with pTa250.15 (Appels and Dvorak 1882). Note that most of the patterns (designated by *letters below* the accession names) share the 0.66-kb promoter fragment (Lassner et al. 1987) but vary in the longer restriction fragments, which range from 1.3 to 3.2 kb and which include the NTS array of 120-bp repeats (Lassner et al. 1987). The major DNA fragments of *Ae. tauschii* pattern *a*, 0.66 and 1.60 kb, are present in the 'Chinese Spring' profile (see also Fig. 3)

The largest number of patterns was found in the Transcaucasian ssp. *tauschii* followed by Transcaucasian ssp. *strangulata* (Table 2). The spacer of a repeat unit of pattern a has been fully sequenced (Lassner et al. 1987). The 0.66-kb fragment comes from the promoter, whereas the 1.60-kb fragment contains an array of seven 120-bp repeats. Variation in the length of the fragment containing the repeated array suggests that there are eight array-length classes which contain 5, 7, 8, 9, 10, 11, 12, and 13 120-bp repeats. These are represented by patterns o, a, h, j, s, t, g, and u, respectively. The most widespread is pattern g, which has 12 120-bp repeats in the array (Table 2). The pattern o with the shortest repeated array was observed only in Turkey and western Iran. The second shortest array (pattern a) was found only in ssp. *strangulata* in Transcaucasia and SE Caspian Iran and ssp. *tauschii* in southwestern (SW) Caspian Iran. Patterns q and s most likely differ from pattern a by an additional *Taq*I site in the promoter (Fig. 2). Only pattern a was found among 254 accessions of *T. aestivum* (Figs. 2 and 3).

Average gene diversity (H) across 55 loci (Table 3) was highest in ssp. *tauschii* in Transcaucasia which parallels the rRNA NTS variation. The average gene diversities of ssp. *tauchii* from the neighboring Turkey and western Iran were significantly lower ($P < 0.01$). Eastward, in the Caspian regions and north-central Iran, H was significantly higher than in western Iran ($P < 0.05$). In Turkmenistan, Afghanistan, and China, values of H were similar to those in Turkey and western Iran ($P > 0.2$). Average gene diversities across the selected 27 loci were universally higher, but their relative magnitudes among the *Ae. tauschii* groups were similar to those based on 55 loci (Table 3). In ssp. *strangulata*, H was higher in SE Caspian Iran than in Transcaucasia ($P < 0.01$).

In the D genome of *T. aestivum*, H was estimated in spelt, ssp. *macha*, and ssp. *vavilovii* across 55 loci and in all six *T. aestivum* groups across the 27 selected loci (Table 3). Across 55 loci, H ranged from 0.01 to 0.05 (Table 3), and the differences were not statistically significant ($P > 0.2$). Across 27 loci, H ranged from 0.00 to 0.07; the lowest was in ssp. *vavilovii* followed by bread wheat (ssp. *aestivum*).

Genetic relationships among *Ae. tauschii* accessions

Nei's genetic distances between the *Ae. tauschii* groups were computed from allelic frequencies at either all 55 loci (70 enzyme × probe combinations) or the selected 27 loci (29 enzyme × probe combinations). In ssp. *tauschii*, the Turkish group was closely related to the neighboring west Iranian group. Both groups were more closely related to the geographically distant ssp. *tauschii* in Afghanistan, Turkmenistan, and China than to ssp. *tauschii* in north-central Iran or SW Caspian Iran (Fig. 4). In ssp. *strangulata*, accessions from SE Caspian Iran were more closely related to the accessions of ssp. *tauschii* in that region and north-central Iran than to the accessions of ssp. *strangulata* from Transcaucasia (Fig. 4). The Transcaucasian ssp. *strangulata* was equally distant to ssp. *tauschii* in the neighboring SW Caspian Iran, SE Caspian Iran, and north-central Iran and ssp. *strangulata* in SE Caspian Iran. The Transcaucasian ssp. *strangulata* was genetically more distant from ssp. *tauschii* in that region than from ssp. *tauschii* in the SW and SE Caspian regions (Fig. 4).

On the basis of these relationships, each group of *Ae. tauschii* accessions could be assigned to one of two distinct genepools (Fig. 4). One genepool, henceforth the "strangulata" genepool, involves ssp. *strangulata* and ssp. *tauschii* from SE Caspian Iran, Transcaucasian ssp. *strangulata*, and ssp. *tauschii* from north-central Iran and SW Caspian Iran (black in Fig. 4). The other genepool, henceforth the "tauschii" genepool, involves ssp. *tauschii* from Transcaucasia, Turkey, western Iran,

Table 2 Frequencies of restriction fragment patterns of the rRNA gene nontranscribed spacers in *Ae. tauschii*

Geographic region and subspecies	Pattern *o*	*a*	*q*	*s*	*t*	*r*	*c*	*j*	*b*	*k*	*g*	*d*	*u*
7. Turkey	1.00												
8. Western Iran	0.67									0.33			
9. Transcaucasia *tausch.*[a]	0.02		0.15	0.04	0.04		0.02	0.22		0.04	0.37	0.03	0.07
	0.03		0.06		0.03		0.03	0.31		0.06	0.38		0.09
10. Transcaucasia *strang.*[a]		0.20				0.36		0.21			0.36	0.02	
		0.16	0.12	0.05	0.02	0.09					0.33		
11. SW Caspian		0.20					0.07	0.27		0.07	0.40		
12. North-central Iran			0.27	0.27							0.36	0.09	
13. SE Caspian *tausch.*							0.16				0.83		
14. SE Caspian *strang.*		0.22						0.50			0.28		
15. Turkmenistan											1.00		
16. Afghanistan											1.00		
17. China								0.15	0.08		0.77		

[a] The top values were computed using the original subspecies allocation of the Transcaucasian *Ae. tauschii* accessions, and the bottom values were computed using the subspecies allocation based on data in Table 4

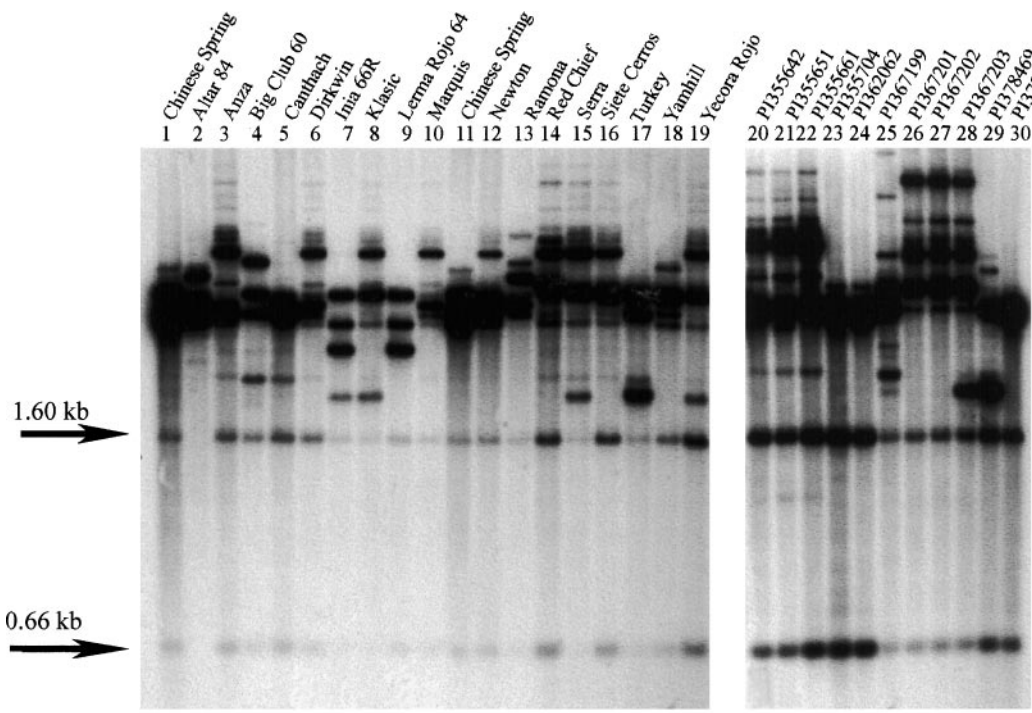

Fig. 3 Autoradiograms of Southern blots of DNAs of a sample of *T. aestivum* accessions. DNAs were digested with the *Taq*I restriction endonuclease and hybridized with pTa250.15. 'Chinese Spring' in *lane 1* serves as a reference (see Fig. 2). DNA fragments of the *Nor3* locus are indicated. DNAs of a selected sample of bread wheat cultivars are in *lanes 1* and *3–19*, and of *spelta* are in *lanes 20–30*; *lane 2* is DNA of *T. turgidum*. *Lanes 20–24* and *29*, *30* are European spelt, and *lanes 25–28* are Afghan spelt. Note that all accessions share the same two 0.66-kb and 1.6-kb fragments characterizing *Nor3* pattern *a* (see Fig. 2). The same result was obtained with all *T. aestivum* accessions investigated in this paper

Afghanistan, Turkmenistan, and China (white in Fig. 4). The "strangulata" genepool is wedged in the "tauschii" genepool (Fig. 4). The genepools do not coincide with the allocation of accessions into subspecies on the basis of morphology.

These findings raised a concern as to the correct assignment of individual accessions to subspecies in Transcaucasia where the two genepools are sympatric. To investigate this question, we used the subspecies *strangulata* accessions from SE Caspian Iran and ssp. *tauschii* accessions from Turkey as outgroups and computed the genetic distances of each Transcaucasian accession to them using variation at 55 loci (Table 4). Of 61 Transcaucasian accessions, 58 showed either a short genetic distance to the ssp. *tauschii* outgroup and a long genetic distance to the ssp. *strangulata* outgroup or vice versa (Table 4). The ratio of the two distances was between 0.2 and 0.7 in the former group of accessions and between 1.8 and 3.8 in the latter group of accessions. Only 3 accessions, AL10/80-1 and AL10/80-2, and KU2829 A, showed intermediate ratios near 1.0. The former 2 accessions originated from a mixed ssp. *tauschii* and ssp. *strangulata* population (V. Jaaska, personal communication). While the

Table 3 Gene diversity among *Ae. tauschii* accessions classified by geographic region.

Species	Subspecies	Geographic region	*H* 55 loci (70 probe × enzyme combination)	*H* 27 loci (29 probe × enzyme combination)
Ae. tauschii	*tauschii*	Turkey	0.07	0.10
	tauschii	Western Iran	0.12	0.17
	tauschii	Transcaucasia	0.29 (0.21)[a]	0.48 (0.32)[a]
	strangulata	Transcaucasia	0.13 (0.13)[a]	0.16 (0.16)[a]
	tauschii	SW Casp. Iran	0.17	0.24
	tauschii	SE Casp. Iran	0.22	0.35
	strangulata	SE Casp. Iran	0.24	0.36
	tauschii	North-central Iran	0.22	0.35
	tauschii	Turkmenistan	0.09	0.14
	tauschii	Afghanistan	0.15	0.18
	tauschii	China	0.10	0.20
T. aestivum	*aestivum*	Western region	–	0.05
	aestivum	Eastern region	–	0.05
	spelta	Europe	–	0.07
	spelta	Asian	0.05	0.06
	vavilovii	Transcaucasia	0.01	0.00
	macha	Georgia	0.05	0.07

[a] Values computed using the subspecies allocation of the Transcaucasian *Ae. tauschii* accessions based on data in Table 4 are in parentheses

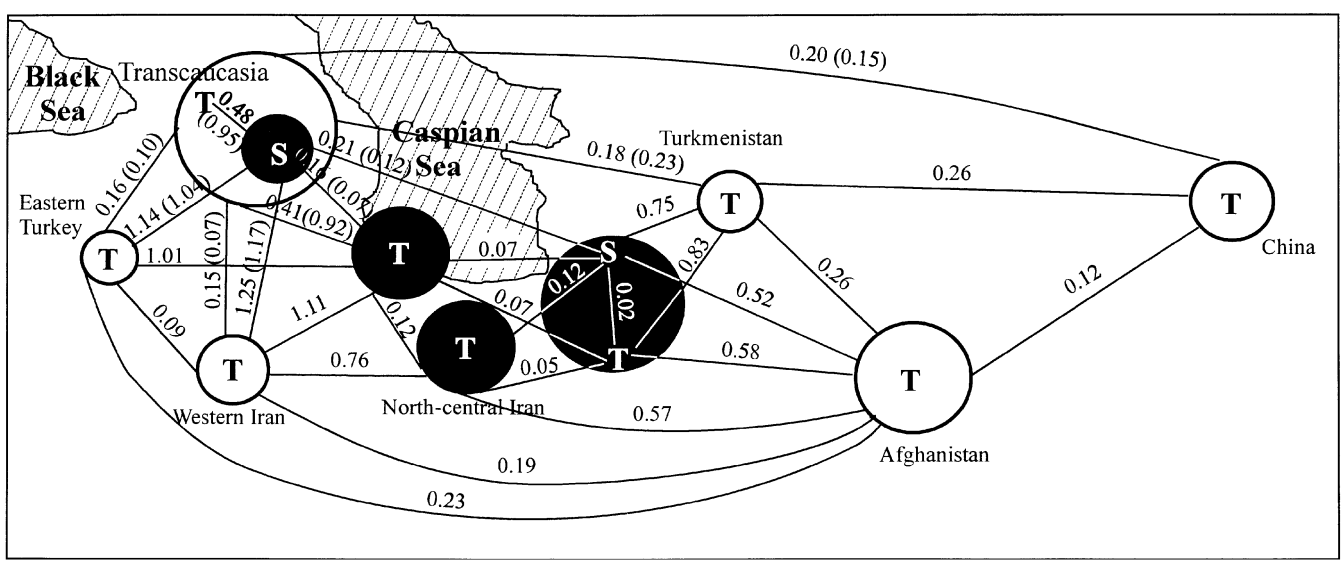

Fig. 4 Schematic representation of genetic and geographic relationships among the *Ae. tauschii* accession groups. Groups classified as ssp. *strangulata* on the basis of morphology are designated *S*, and those classified as ssp. *tauschii* are designated *T*. *Black circles* represent the ssp. *strangulata* genepool, and the *white circles* represent the ssp. *tauschii* genepool. Critical distances between groups based on genetic variation at the 27 selected loci are shown. Distances computed using reallocated Transcaucasian accessions are in *parentheses*. The *sizes* of the *circles* approximate the average gene diversities of the groups (Table 3). The location of the groups on the map only approximately reflects their geographic location. For more accurate locations see Fig. 1

allocation into the subspecies on the basis of morphology agreed with genetic distances to the outgroups for all accessions supplied by V. Jaaska (Tables 1 and 4), about a third of those assigned to ssp. *tauschii* in the Kihara et al. (1965) collection showed a genetic relationship characteristic of ssp. *strangulata*, not ssp. *tauschii* (Table 4). A majority of these accessions came from Azerbaijan and 2 from Georgia. Those accessions (identified by superscript b in Table 1) were reallocated to the "strangulata" genepool. The frequencies of *Nor3* restriction fragment patterns in Transcaucasia (Table 2), average gene diversity (Table 3), and genetic distances (Fig. 4) were computed again and are shown in Tables 2 and 3 and Fig. 4, respectively.

Phenograms using the 27 and 55 loci were constructed using genetic distances based either on the original subspecies allocation of the Transcaucasian accessions or on reallocated Transcaucasian accessions (Fig. 5). The topology and the lengths of the branches of the

Table 4 Genetic distances of individual accessions from Transcaucasia to ssp. *tauschii* accessions in Turkey (T) and ssp. *strangulata* accessions in southeastern Caspian Iran (S)

Accessions	T	S	Ratio T/S	Accessions	T	S	Ratio T/S
AL9/78-1, 2, 3, 4, 6, 7, 10	0.14–0.20	0.30–0.38	0.4–0.6	AL7/80-3, 5, 9	0.45–0.64	0.19–0.30	1.8–2.4
AL10/80-1, 2	0.60–0.66	0.53–0.65	1.0	AL8/78-2, 4, 5	0.52–0.55	0.20–0.30	1.8–3.0
KU2116	0.15	0.35	0.4	AL11/80-1, 2, 3, 4	0.53–0.54	0.18-0.20	2.7–3.0
KU2120	0.10	0.30	0.3	AL10/80-3	0.56	0.25	2.2
KU2121	0.12	0.36	0.3	AL46/77- 3, 4	0.58	0.19–0.22	2.6–3.0
KU2122	0.20	0.29	0.7	K1574/72-1, 9	0.53–0.54	0.17–0.18	2.9–3.2
KU2141	0.14	0.33	0.4	KU2110	0.57	0.20	2.9
KU2142	0.06	0.35	0.2	KU2111	0.52	0.16	3.2
KU2144	0.20	0.33	0.6	KU2112	0.54	0.18	3.0
KU2145	0.10	0.35	0.3	KU2117	0.54	0.18	3.1
KU2148	0.18	0.40	0.5	KU2118	0.52	0.16	3.2
KU2151	0.13	0.29	0.5	KU2123	0.54	0.14	3.8
KU2809	0.16	0.30	0.5	KU2804	0.48	0.18	2.7
KU2810	0.18	0.29	0.6	KU2806	0.52	0.18	2.9
KU2814	0.16	0.29	0.5	KU2808	0.57	0.19	3.0
KU2816	0.15	0.30	0.5	KU2811	0.48	0.13	3.7
KU2821	0.09	0.32	0.3	KU2827	0.49	0.17	2.9
KU2822A	0.17	0.32	0.5	KU2828	0.48	0.14	3.4
KU2822B	0.21	0.32	0.6				
KU2823	0.11	0.35	0.3				
KU2824A	0.15	0.30	0.5				
KU2824B	0.10	0.31	0.3				
KU2826	0.14	0.38	0.4				
KU2828	0.13	0.36	0.4				
KU2829A	0.42	0.38	1.1				
KU2829B	0.11	0.35	0.3				
KU2832	0.23	0.42	0.5				

phenograms based on the two allocations of Transcaucasian accessions were essentially identical except for that the Transcaucasian ssp. *tauschii* was widely separated from the Transcaucasian ssp. *strangulata* and clustered with the accessions from Turkey, western Iran, Afghanistan, Turkmenistan, and China in the phenogram based on the reallocated accessions. Additionally, the reallocation of accessions revealed that there is almost complete allelic differentiation between the 2 subspecies in Transcaucasia at a number of loci. At *Xbcd1302*, *Xcdo749*, *XGsp*, *Xpsr371-6D*, and *Xpsr901-2D,* an allele that was fixed in ssp. *strangulata* (frequency $f = 1.0$) was near elimination ($f \leq 0.05$) in ssp. *tauschii*. Allelic differentiation of this magnitude was not found at any locus in SE Caspian Iran.

Kihara et al. (1965) reported var 'meyeri' only in SW Caspian Iran. Of 15 accessions from this region used in this study, 6 were classified by Kihara et al. (1965) as var 'meyeri' and 9 as var 'typica'. Genetic distance between these two groups was 0.03, using the 27 selected loci. Genetic distances of var 'meyeri' and var 'typica' in SW Caspian Iran to Transcaucasian ssp. *strangulata* were 0.09 and 0.07, respectively. Genetic distances of var 'meyeri' and var 'typica' in SW Caspian Iran to Transcaucasian ssp. *tauschii* were 0.89 and 0.95, respectively (27 loci). Clearly, there is virtually no genetic difference between var 'typica' and var 'meyeri' in SW Caspian Iran, and both belong to the "strangulata" genepool.

Genetic relationships between *Ae. tauschii* and *T. aestivum*

Because the 55 loci were investigated in only 1 accession, bread wheat as a group was excluded from comparisons involving 55 loci. To assess the relationships of the *T. aestivum* D genome with the *Ae. tauschii* subspecies, we combined accessions of ssp. *strangulata* from Transacucasia and SE Caspian Iran. For ssp. *tauchii*, accessions from only Transcaucasia, the Caspian regions, and north-central Iran were used in order to employ accessions from approximately the same geographic area for both subspecies. Variation at 27 loci was used. The distance between *T. aestivum* and ssp. *strangulata* was 0.33 and 0.29 and between *T. aestivum* and ssp. *tauschii* was 0.41 and 0.75, using the original and revised allocation of *Ae. tauschii* accessions into subspecies, respectively. Hence, irrespective of which classification of accessions was used, the *T. aestivum* D genome was more closely related to ssp. *strangulata* than to ssp. *tauschii*. The same result was obtained for each of the six individual groups of *T. aestivum* (not shown).

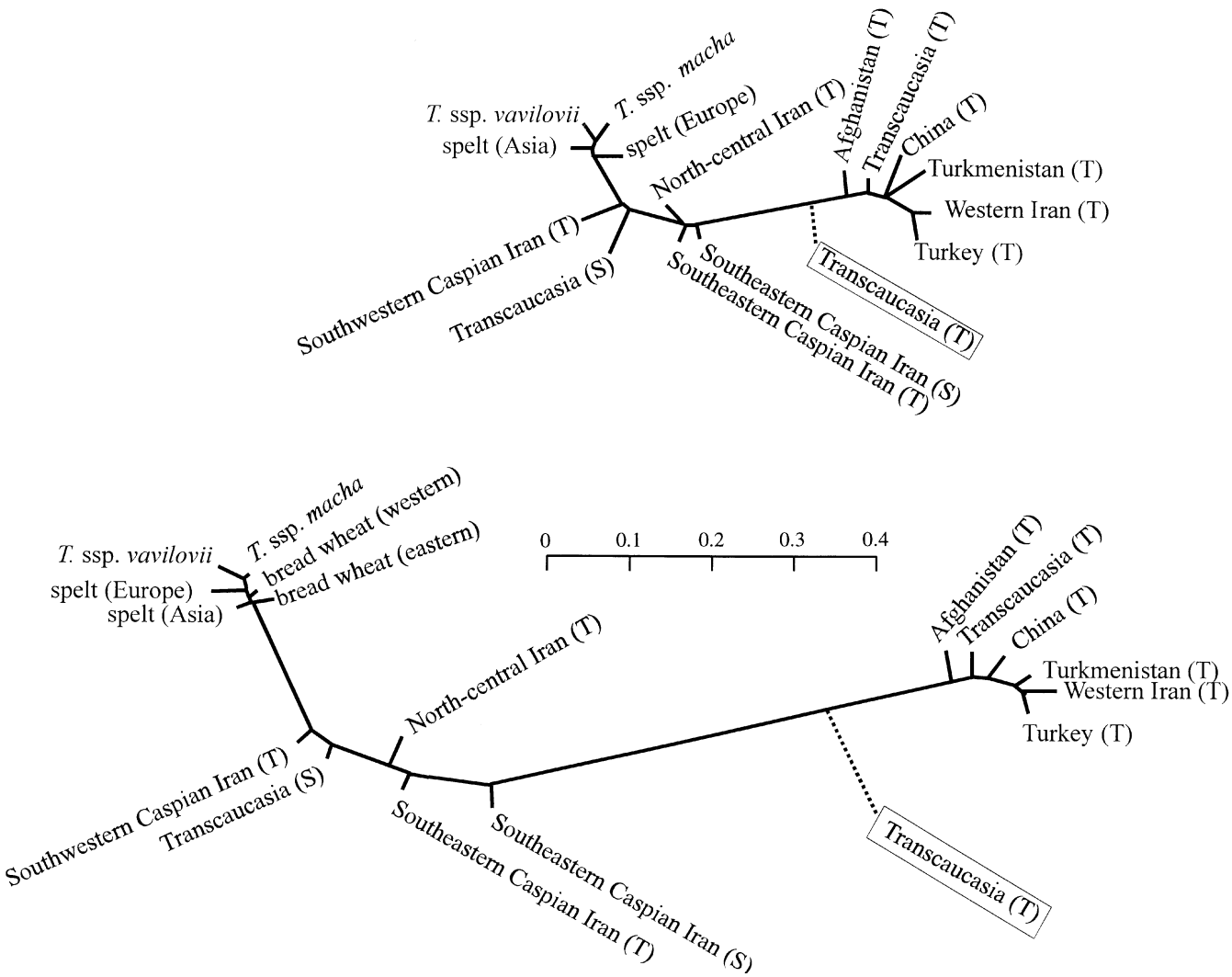

Fig. 5 Redrawn phenograms produced by the GDA computer program using the neighbor-joining method. The *upper* phenogram was constructed from genetic distances based on variation in 70 enzyme × probe combinations, and the *lower* one was constructed from genetic distances based on the selected 27 loci (29 enzyme × probe combinations). Genetic distances computed using reallocated Transcaucasian accessions into subspecies were used. The position of the Transcaucasian ssp. *tauschii* based on the original accession allocation into subspecies is boxed. A genetic distance (*D*) scale is between the phenograms. *Aegilops tauschii* populations of accessions identified as ssp. *tauschii* on the basis of morphology are indicated by (*T*), and those identified as ssp. *strangulata* are indicated by (*S*). The branch lengths based on the 55 loci are shorter than those based on the 27 selected loci because the former contained a number of monomorphic or nearly monomorphic enzyme × probe combinations

A closer relationship between *T. aestivum* and ssp. *strangulata* than between *T. aestivum* and ssp. *tauschii* is also indicated by allele sharing by *T. aestivum* and *Ae. tauschii*. The *Xcdo749*, *Xpsr371-6D*, and *Xpsr901-2D a* alleles fixed in ssp. *strangulata* and nearly eliminated from ssp. *tauschii* in Transcaucasia (see the previous section) were present in all six groups of *T. aestivum* (Table 5). The *Xbcd1302 a* allele, which is the most frequent *Xbcd1302* allele in *T. aestivum*, was not found in *Ae. tauschii* (Table 5). Except for 1 accession, allele *c*, which is rare in *T. aestivum* (Table 5), was found only in the "strangulata" genepool in Transcaucasia.

On the geographic region basis, all groups of *T. aestivum* showed the closest relationship to the group of accessions from SW Caspian Iran and ssp. *strangulata* from Transcaucasia (Fig. 5). This was true irrespective of which allocation of Transcaucasian accessions was used.

The Transcaucasian accessions of the "strangulata" genepool (Table 4) were divided into four geographic regions, Georgia, Armenia, Nakhichevan, and Azerbaijan, and the genetic distances of these groups, in addition to those of the remaining groups of the "strangulata" genepool, to *T. aestivum* were compared (Table 6). The D genomes of all *T. aestivum* groups

Table 5 Alleles[a] in *Ae. tauschii* and the D genome of *T. aestivum* and polymorphism sharing between *T. aestivum* and *Ae. tauschii*

Accessions	*Xmwg-2031*	*Xcdo-1400*	*Xpsr371-6D*	*XGlu1* (*Eco*RV)	*Xpsr899*	*Xbcd-1302*	*XEsi3*	*Xpsr666* (*Xba*I)	*Xpsr-102*	*Xpsr-901-2D*	*Xpsr-928*	*Xwg-644*	*XGsp*	*Xcdo-749*
Ae. tauschii	*a*, *b*	*b*, *c*, *d*, *e*	*a*, *b*, *c*	*a*[c], *b*, *c*, *d*	*a*, *b*, *c*, *d*, *e*, *f*, *g*	*b*, *c*, *d*, *e*, *f*	*b*, *c*, *d*	*a*, *b*, *c*	*a.b*	*a*, *b*	*a*, *b*	*abc*	*b*, *c*, *e*, *f*	*a*, *b*, *c*, *d*
Wheat west reg.	*a*	***a***[b], *c*	*a*	*a*, *b*	*a*, *f*, ***h, i***	***a, g***	*d*	*a*	*a*, ***c***	*a*, *b*	*a*, *b*	*a*	***a***	*a*
Wheat east reg.	*a*	***a***, *c*	*a*, ***d, e***	–	*a*, *f*	***a***, *c*	***a***, *d*	*a*, *b*	*a*, ***d***	*a*, *b*	*a*, *b*	*a*	***a***	*a*
ssp. *macha*	*a*	*c*	*a*	*a*, *b*	*a*, *f*, ***j, k***	***a***	***a***, *d*	*a*	*a*, ***d***	*a*, *b*	*a*, *b*	*a*	***a***	*a*, ***e***
Spelt (Asia)	*a*, ***c***	*c*	*a*	*a*	*a*, *f*	***a***, *c*	*d*	*a*, *b*	*a*, ***d***	*a*	*a*, *b*	*a*	***a***	*a*
Spelt (Europe)	*a*	*c*, ***f***	*a*	*b*	*a*, *f*	***a***	*d*	*a*	*a*, ***c, d***	*a*, *b*	*a*	*a*, ***b***	***a***	*a*
ssp. *vavilovii*	*a*	*c*	*a*	*a*	*a*, *f*	***a***	***a***, *d*	*a*	***d***	*a*	*a*	*a*	***a***	*a*

[a] DNAs digested with *Dra*I were used for all but 2 loci
[b] *T. aestivum* alleles which were not found in *Ae. tauschii* are in bold.
[c] *Ae. tauschii* alleles shared with *T. aestivum* are underlined

Table 6 Genetic distances between *T. aestivum* (1 through 6) and *Ae. tauschii* (7 through 11) and between *T. aestivum* and *Ae. tauschii*[a]

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Bread wheat western reg.	–	–	–	–	–	–	–	–	–	–	–	–	
2. Bread wheat eastern reg.	< 0.01	–	–	–	–	–	–	–	–	–	–	–	–
3. ssp *macha*	0.04	0.04	–	0.01	0.04	0.02	0.15	0.13	0.18	0.16	0.16	0.21	0.20
4. Spelt Asia	< 0.01	0.01	0.04	–	0.04	0.03	0.15	0.12	0.18	0.15	0.15	0.21	0.20
5. Spelt Europe	0.03	0.03	0.06	0.04	–	0.04	0.18	0.15	0.20	0.18	0.18	0.21	0.20
6. ssp. *vavilovii*	0.05	0.05	0.02	0.06	0.06	–	0.16	0.14	0.19	0.17	0.17	0.22	0.26
7. ssp. *strangulata* Georgia	0.27	0.27	0.30	0.29	0.33	0.33	–	0.04	0.02	0.03	0.09	0.06	0.05
8. ssp. *strangulata* Armenia	0.22	0.21	0.22	0.21	0.24	0.25	0.04	–	0.04	0.02	0.08	0.08	0.06
9. ssp. *strangulata* Nakhich.	0.33	0.32	0.33	0.32	0.35	0.36	0.05	0.05	–	0.02	0.12	0.13	0.07
10. ssp. *strangulata* Azerbaij.	0.29	0.29	0.29	0.28	0.32	0.32	0.01	0.02	0.03	–	0.08	0.09	0.06
11. SW Caspian Iran	0.21	0.21	0.20	0.21	0.24	0.24	0.07	0.08	0.13	0.08	–	0.06	0.10
12. ssp. *stran*. SE Caspian Iran	0.38	0.37	0.38	0.37	0.39	0.41	0.07	0.12	0.16	0.13	0.07	–	0.05
13. North-central Iran	0.36	0.36	0.36	0.36	0.38	0.40	0.07	0.07	0.08	0.07	0.12	0.05	–

[a] Distances based on 55 loci are above the diagonal and those based on 27 loci are below the diagonal. The subspecies allocation of *Ae. tauschii* accessions is based on data in Table 4

were equidistant to the groups of accessions from Armenia and SW Caspian Iran when 27 loci were used. Only spelt, ssp. *macha*, ssp. *vavilovii* and 'Chinese Spring' (ssp. *aestivum*) were investigated with 55 loci. On the basis of variation at 55 loci, all were more closely related to *Ae. tauschii* accessions from Armenia than to those from the remaining groups of the "strangulata" genepool. Most of the Armenian accessions were collected in the vicinity of Jerevan and the Razdan River (V. Jaaska, personal communication and Kihara et al. 1965). Similar relationships were found when comparisons were made between individual accessions of *T. aestivum* and *Ae. tauschii*. With 55 loci, 21 out of 24 accessions of ssp. *macha*, ssp. *vavilovii*, spelt, and cv 'Chinese Spring' were most closely related to an accession from population AL8/78 collected near the Razdan River. Using 27 loci, a majority of *T. aestivum* accessions were most closely related to accessions from SW Caspian Iran.

Of the 55 *T. aestivum* D-genome loci 14 were polymorphic. At 5 loci, polymorphisms in *T. aestivum* paralleled the same polymorphisms in *Ae. tauschii* and at 9 loci, polymorphisms were unique to *T. aestivum*; a total of 18 alleles not encountered in *Ae. tauschii* were found in *T. aestivum* (Table 5).

Genetic relationships within *T. aestivum*

In comparisons based on 27 loci, most closely related were the two groups of bread wheat (Table 6). In turn they both were most closely related to the Asian spelta and the least to ssp. *macha* and *ssp. vavilovii*. The latter 2 subspecies were closely related to each other.

Examination of the distribution of *T. aestivum* polymorphisms among the six *T. aestivum* groups showed little differentiation among the groups (Table 5). For example, the *Dra*I *a* alleles at the *XGsp* and *Xbcd1302* loci were not found in *Ae. tauschii* but were present in all *T. aestivum* groups (Table 5). Likewise, the *f* allele at the *Xpsr899* locus, which is rare in *Ae. tauschii*, was observed in high frequencies in all groups (frequency ranged from 0.30 in ssp. *macha* to 1.0 in the Afghan spelt); a sole exception was the European spelt in which it was not found. The *Glu1 Eco*RV *b* allele is shared by the European spelt, bread wheat, and ssp. *macha*. The *d* allele at *Xpsr102*, which was not found in *Ae. tauschii*, was found in all groups of wheat except for the group of western cultivars investigated here. Both alleles at the *Xpsr928* locus contributed by *Ae. tauschii* were present in bread wheat, ssp. *macha*, and the Asian spelt. These and other examples in Table 5, as well as small genetic distances among the D genomes of all forms of *T. aestivum* (Fig. 5 and Table 6) show that the D-genomes of *T. aestivum* are not markedly differentiated from each other.

Discussion

The western *Ae. tauschii* groups (Turkey and western Iran) are more closely related to the eastern groups (Afghanistan, Turkmenistan, and China) than to those from the geographically close SW Caspian Iran and north-central Iran. A similar finding was reported by Lubbers et al. (1991). To explain these curious relationships we hypothesize that the modern *Ae. tauschii* populations originated by expansion of two geographically isolated subspecies, ssp. *strangulata* in a coastal region of eastern Caspian Iran and ssp. *tauschi* in an inland area in northwestern Iran. Expansion of the distribution of ssp. *tauschii* preceding that of ssp. *strangulata* would lead to the spread of essentially pure ssp. *tauschii* populations both westward to Turkey and eastward to Afghanistan, Turkmenistan, Pakistan, Tadjikistan and China. Expansion of the distribution of ssp. *strangulata* would cause gene flow between the two subspecies in the Caspian region and north-central Iran. This gene flow would generate discontinuity of the pure ssp. *tauschii* in Iran.

Genetic distances (Fig. 4) and lack of allelic differentiation between the subspecies provide clear evidence for gene migration between the two subspecies in Iran. Kihara et al. (1965) reported hybridization and morphologically intermediate forms between ssp. *strangulata* and ssp. *tauschii* in Iran. In Transcaucasia, the 2 subspecies were reported to be isolated from each other (Jaaska 1981). In agreement with Jaaska's (1981) findings, almost complete differentiation between the subspecies was found at 5 of the 55 loci in Transcaucasia. However, even in Transcaucasia the subspecies are not completely isolated from each other. This is indicated by the observation that 3 of the 61 Transcaucasian accessions showed intermediate relationships to the ssp. *strangulata* and ssp. *tauschii* outgroups; 2 originated from a sympatric population containing both subspecies (V. Jaaska, personal communication). It is possible that the relatively greater isolation of the subspecies in Transcaucasia than in SE Caspian Iran reflects more recent sympatry between the two subspecies in Transcaucasia. Whether or not geographic and environmental factors of Transcaucasia contribute to the maintenance of genetic differentiation between the "strangulata" and "tauschii" genepools is not known.

The model of the evolution of the 2 subspecies proposed here satisfactorily accounts for the apparent disjoined distribution of ssp. *strangulata* between Transcaucasia and SE Caspian Iran. Although accessions from the intervening SW Caspian region are classified morphologically as ssp. *tauschii*, they belong to the "strangulata" genepool and likely originated by northward gene migration from ssp. *tauschii* into ssp. *strangulata*. Lubbers et al. (1991) reported that var 'meyeri', which Kihara et al. (1965) found only in this

geographic region, was closer to ssp. *strangulata* than to ssp. *tauschii* var 'typica' and var 'anathera' and concluded that var 'meyeri' belongs to ssp. *strangulata*. While this conclusion superficially agrees with findings made here, it must be emphasized that var. *meyeri* and var 'typica' are not differentiated from each other in SW Caspian Iran and show the same genetic distance to ssp. *strangulata*. The entire SW Caspian population belongs to the "strangulata" genepool, not just var *meyeri*.

Allozyme evidence on the origin of *T. aestivum* (Nishikawa 1974; Nishikawa et al. 1980; Jaaska 1978, 1980, 1981; Nakai 1979; Lagudah et al. 1991) has suggested that the source of the wheat D genome was ssp. *strangulata*. Variation in rRNA gene NTS and high-molecular-weight glutenin subunits provided additional evidence in favor of ssp. *strangulata* being the source of the wheat D genome (Lagudah et al. 1991; Dvorak et al. 1998b; present data). Genetic distances reported here were overall closer between the wheat D genome and ssp. *strangulata* than between the wheat D genome and ssp. *tauschii*. Subspecies *tauschii* is heterogeneous and involves populations belonging to both the "tauschii" and "strangulata" genepools. It is, therefore, more correct to consider the "strangulata" genepool as the source of the *T. aestivum* D genome rather than the botanical ssp. *strangulata*.

Jaaska (1981) placed the origin of *T. aestivum* to Transcaucasia and Nishikawa et al. (1980) to SE Caspian Iran, although Nishikawa et al. (1980) did point out that SW Caspian region could also be involved. In the materials employed by Jaaska (1981), most of the ssp. *strangulata* accessions came from Transcaucasia, whereas in those employed by Nishikawa et al. (1980) most came from SE Caspian Iran. Thus, the sources of materials might have affected conclusions on the geographic place of the origin of *T. aestivum*.

The study reported here included accessions collected both by Kihara et al. (1965) and Jaaska (1995). Accessions of the "strangulata" genepool from Armenia and those collected by Kihara et al. (1965) in the coastal SW Caspian Iran between Chalus and Astara (classified as ssp. *tauschii*) show the closest genetic relationships to the D genome of *T. aestivum*. Alleles at the highly variable *Nor3* and *Glu1* loci in the *T. aestivum* D genome also point to the "strangulata" genepool in Transcaucasia and SW Caspian Iran as the likely source of the *T. aestivum* D genome. The *Nor3a* allele, for which *T. aestivum* is monomorphic (Lagudah et al. 1991 and present data), occurs only in the "strangulata" genepool and is present at a high frequency in both regions. Accessions from SW Caspian Iran are characterized by a high frequency of the *Glu1* allele encoding high-molecular-weight glutenin subunit 5 and the highest frequency of the *Glu1* haplotype encoding the pair of high-molecular-weight glutenin subunits 5 + T5 (Dvorak et al. 1998b). This haplotype appears to be ancestral to wheat haplotype *Glu1d* (Dvorak et al. 1998b). The *Glu1a* haplotype encoding the high-molecular-weight glutenin subunits 2 + 12 was absent from the accessions collected in SW Caspian Iran investigated here but was present in the "strangulata" genepool in Transcaucasia (Dvorak et al. 1998b). The genepool of the *T. aestivum* D genome is a composite of several sources (Dvorak et al. 1998b), and their representation in the genepool of modern *T. aestivum* has likely been modified by selection. Additionally, it is unclear to what extent the present-day "strangulata" genepool has been affected by agriculture in Transcaucasia and Caspian Iran. It is conceivable that the spread of wheat cultivation could cause migration of *Ae. tauschii* genes and modification of the genetic structure of *Ae. tauschii* populations. Although evidence points to Armenia as a likely birthplace of *T. aestivum*, it seems unrealistic to exclude other areas in Transcaucasia and SW Caspian Iran from playing a role in the evolution of *T. aestivum*.

Whether spelt or other hulled wheats are ancestral to free-threshing wheat, as argued by McFadden and Sears (1946), or some or all are actually derived from hybridization of a free-threshing hexaploid wheat with hulled tetraploid wheat (Tsunewaki 1968; Liu and Tsunewaki 1991; Nesbitt and Samuel 1996) cannot be resolved from these data. All *T. aestivum* groups and individual accessions showed the same relationship to *Ae. tauschii*. Many of the alleles found only in the *T. aestivum* D genome, and likely originating by mutations in *T. aestivum*, were shared by several or all *T. aestivum* groups. The same is true for the 5 polymorphisms shared by *T. aestivum* and *Ae. tauschii*. These facts strongly suggest that the modern forms of *T. aestivum* share a common D-genome genepool and argue against independent evolution of any of the *T. aestivum* forms, as has been suggested for ssp. *macha* (Dekaprelevich 1961; Swaminathan 1966) or some of the Chinese wheats (Yen et al. 1983).

There are two basic scenarios by which a single *T. aestivum* D-genome genepool could have been formed. Several founding amphiploids could have originated in different geographic regions, and hybridization, recombination, selection, and genetic drift could have lead to the evolution of a single gene pool. Alternatively, a single amphiploid could have originated and founded a hexaploid population. Plants in this hexaploid population could have hybridized with *Ae. tauschii* and the hybrids could have formed a bridge for gene flow from *Ae. tauschii* to *T. aestivum*. This scenario seems unlikely. While hybridization between tetraploid wheat and *Ae. tauschii* is easy and fertile amphiploids are produced by self-pollination of triploid F_1 hybrids due to high production of unreduced gametes (Kihara et al. 1950), hybridization between hexaploid wheat and *Ae. tauschii* is difficult and the production of hybrid plants usually requires embryo rescue. Therefore, the recurrent appearance of hexaploid amphiploids in the fields

of tetraploid wheat or mixed tetraploid/hexaploid wheat was a likely source of gene flow from *Ae. tauschii* to the hexaploid genepool (for additional discussion see Dvorak et al. 1998b). A recurrent origin of allopolyploids from the same parental combination has been documented in other plant groups (e.g., Ownbey 1950; Soltis et al. 1995 and references therein).

If the genepool of the wheat D genome originated by multiple hybridization events involving *Ae. tauschii*, a relevant concern is why so few cases of shared polymorphism between wheat and *Ae. tauschii* have been encountered. A glaring example is the *Nor3* locus, which is highly polymorphic in *Ae. tauschii* but monomorphic in *T. aestivum* for a rare *Ae. tauschii* pattern. This dilemma can be accounted for if it is assumed that the differentiation of the *T. aestivum* genepool into the present-day forms was preceded by a significant evolution of the genepool. During that period, some of the polymorphisms may have been lost from the genepool and new polymorphisms may have evolved. Another factor that may have played a role is that not all amphiploids have contributed equally to the genepool. If a single amphiploid established the initial hexaploid, alleles contributed by subsequent amphiploids would have a general stochastic tendency to be lost. This is particularly true after the establishment of free-threshing wheat from which hulled plants would tend to be eliminated during threshing (Dvorak et al. 1998b).

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