Natural variation of morphological traits in wild wheat progenitor *Aegilops tauschii* Coss.

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Aegilops tauschii Coss. (syn *Ae. squarrosa* L.) is a wild diploid wheat species. It has a wide natural species range in central Eurasia, spreading from northern Syria and Turkey to western China. *Ae. tauschii* is known as the D genome progenitor of hexaploid bread wheat. The genealogical and geographical structure of variation of morphological traits was analyzed using a diverse array of 205 sample accessions that represented the entire species range. In total, 27 traits, including anther and pistil shape and internode length, were examined in this study. Large-scale natural variation was found for all examined traits. Geographically, significant longitudinal clines were detected for anther size, internode length and spike size and shape. Anthers tended to be small in accessions from the eastern region. Internodes also tended to be short, whereas spikes tended to be long in accessions from the eastern region. Spikelet density per spike tended to be high in the eastern habitats. In the process of west-to-east dispersal, *Ae. tauschii* underwent extensive morphological, genetic and ecological diversification that produced the variation seen among today's natural populations.

Key Words: diversification, geographical cline, morphology, variation, wild wheat.

Introduction

Aegilops tauschii Coss. (syn. *Ae. squarrosa* L.), a diploid self-pollinating goatgrass, is the D genome donor of common wheat (Kihara 1944, McFadden and Sears 1944). The genome of *Ae. tauschii* was brought into that of common wheat through a spontaneous species cross with tetraploid emmer wheat and subsequent amphidiploidization about 8,000 years ago (Nesbitt and Samuel 1996). Habitats of *Ae. tauschii* are widely distributed from north Syria and Turkey to western China in Eurasia. *Ae. tauschii* populations, which maintain large diversity at the nucleotide sequence level (Caldwell *et al.* 2004), provide a useful source for common wheat breeding. In fact, some studies have reported higher levels of genetic variability of glutenin subunits and gliadin in *Ae. tauschii* than in the D genome of common wheat (Gianibelli *et al.* 2001, 2002, Yan *et al.* 2003a, 2003b, Giles and Brown 2006). *Ae. tauschii* has been used by wheat breeders to integrate pest resistance traits into wheat cultivars (Zhu *et al.* 2005).

The subspecies classification of *Ae. tauschii* is still under discussion. Two subspecies have been described, *Ae. tauschii* Coss. subspecies *tauschii* (syn. *Ae. squarrosa* L. subspecies *eusquarrosa* Eig) and *Aegilops tauschii* Coss. subspecies *strangulata* (Eig) Tzvel (syn. *Ae. squarrosa* L. subspecies

strangulata Eig) based on spikelet morphology, whereas the typical forms of subspecies *tauschii* and *strangulata* are connected by a continuous range of intermediate forms (Dudnikov 1998). Some recent reports have also shown difficulty in distinguishing the two subspecies based on molecular markers and suggested a high level of gene flow between them (Dvorak *et al.* 1998, Dudnikov and Kawahara 2006, Saeidi *et al.* 2006). Two characteristics that distinguish the subspecies have been described: one is glume width to rachis segment width ratio, another is allozyme variation at the *Acp1* locus (Dudnikov 2000). Our previous study showed that these subspecies form separate clusters in a graph of the first two components from principal component (PC) analysis based on nine spikelet traits and suggested that subspecies *strangulata* diverged from an ancestor that carried a specific chloroplast DNA type found in the western habitats. Moreover, natural variation in spikelet-related traits showed significant longitudinal and latitudinal clines for spikelet size (Matsuoka *et al.* 2009). Latitudinal and longitudinal clines were also found for natural flowering time variation. The early-flowering accessions spread mainly in eastern habitats such as Afghanistan and Pakistan, implying that the early-flowering phenotype contributed to eastward dispersal and adaptation to these habitats in *Ae. tauschii* (Matsuoka *et al.* 2008).

Relatively little is known about genetic and phenotypic characteristics of the eastern and southern populations because previous studies have mainly used accessions from the Transcaucasus and northern Iran, the center of the species'

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diversity. The eastern and southern populations (i.e., those from Afghanistan, Pakistan, Central Asia and China), however, have the potential to provide materials suitable for study of the mechanisms underlying *Ae. tauschii*'s wide ecological adaptation. Such studies may help elucidate the expanded distribution of *Ae. tauschii* eastward from Transcaucasus to China. In this study, we used 205 *Ae. tauschii* accessions covering the species range and performed a common garden experiment to analyze the natural variation of morphological traits. PC analysis of the morphological traits and phylogenetic network based on chloroplast DNA variation provided information about subspecies formation and the process of the species' west-to-east dispersal. The implications of these findings for intraspecific morphological diversification are discussed.

Materials and Methods

Plant materials

In total, 205 accessions of *Ae. tauschii* were used in this study (Table 1). Their passport data including geographical coordinates have been provided in previous reports (Matsuoka *et al.* 2005, 2007, 2008). This sample set consisted of 199 accessions representing the entire natural habitat range (Fig. 1) and six accessions (AT 47, AT 55, AT 60, AT 76, AT 80, and PI 508264) representing adventive populations in the Shaanxi and Henan provinces of China. When geographical coordinates of sampling sites were not available, we estimated latitude and longitude by means of Kashmir 3D software (<http://www.kashmir3d.com/>) on scanned paper maps (scales 1 : 4,000,000–1 : 1,000,000) based on locality information. For four accessions (CGN 10731, CGN 10732, CGN 17333, and CGN 10734), geographical coordinates were not estimated due to lack of locality information. In the original collection, most of the accessions were not obtained by population sampling. For each accession, we used seeds propagated from a single plant by selfing.

Chloroplast DNA haplogroups (HGs) of the 205 accessions were also previously determined based on data from biallelic single nucleotide polymorphism and minisatellite sites that were found in the flanking regions of chloroplast microsatellite loci (Matsuoka *et al.* 2005, 2007, 2008, 2009). Clustal W software ver. 1.8 (Thompson *et al.* 1994) was used for alignment of the chloroplast DNA sequences, and Network software ver. 4.112 (<http://www.fluxus-engineering.com/>) (Bandelt *et al.* 1995) was used for reduced median network construction. On the basis of the network topology, four intraspecific lineages were defined as follows: the HG7 lineage (HG7 and its derivatives HG2, HG4, HG5, HG6, HG8, HG10, HG11, HG12, HG13 and HG14), the HG9 lineage (HG9 and its derivatives HG1, HG3 and HG18), the HG16 lineage (HG16 and its derivative HG15), and the HG17 lineage (HG17) (Matsuoka *et al.* 2008, 2009). The major HG lineage information of the 205 accessions is represented in Table 1.

Analysis of morphological variation

Seeds of the sample accessions were sown in November 2004. Plants were grown in a field of Kobe University, and the accessions were arranged in the field using a randomized design. For each accession, a single healthy plant was chosen for analysis of morphological variation. All morphological traits were measured using the three tillers of each plant that headed earliest, and the trait averages and standard deviations were calculated.

In total, 27 traits were studied, and their abbreviations are listed in Table 2. Out of them, nine traits related to the spikelet morphology, i.e. spikelet length (SpL), empty glume length (GL), empty glume width (GW), empty glume thickness (GT), empty glume height (GH), lemma length (LL), lemma width (LW), palea length (PaL), and palea width (PaW), were previously studied (Matsuoka *et al.* 2009), and their data was used in this study. Trait measurements were done using the first, second, and third culms and spikes. The first and second florets of the central spikelet were used for estimation of the pistil and anther traits before anthesis. Awn length was measured at the three positions, the first florets of top and central spikelets (TAL and AwL, respectively) and the fourth floret of the central spikelet (AwL4). Internode length of spikelet (ILSp) was estimated as an average of internode lengths before and after the central spikelet.

The morphological trait data were statistically analyzed using JMP software ver. 5.1.2 (SAS Institute). Cluster analysis among the traits was conducted by the clustering program (<http://www.tinet.org/~debb/UPGMA/>) that calculated all the Pearson correlation coefficients between pairs of sets of variables, transformed these coefficients into distances and made a clustering using the unweighted pair group method with arithmetic mean (UPGMA) algorithm (Garcia-Vallve *et al.* 1999).

Results

Natural variations in 27 morphological traits

In total, 27 morphological traits were measured before anthesis. Large natural variations were found for all examined traits, especially spike-, spikelet- and floret-related traits (Table 2). In most examined traits, the maximum values were two to three times the minimum values. Two awn length traits, AwL and AwL4, exhibited large standard deviations (Table 2). Of the 27 examined traits, 19 (SL, NSp, GL, GW, GT, GH, AwL, LL, LW, AnL, AnT, PiL, PiW, PiT, PaL, PaW, MCL, MCT, and IL4) showed a significant difference in means between the subspecies (Student's *t* test, Table 2).

Among the examined traits, the highest correlations were observed among the glume, lemma and palea traits, and the spikelet length (SpL) was closely related to the lemma and palea lengths (LL and PL) (Fig. 2A). Cluster analysis of the examined morphological traits showed strong association among the spike, spikelet and floret traits (Fig. 2B). Anther and pistil sizes were highly associated with spikelet and

Table 1. Strain numbers and sources of the *Ae. tauschii* accessions used in this study

Afghanistan (n=35)
KU-2010 ^a , KU-2012 ^a , KU-2016 ^a , KU-2018 ^c , KU-2022 ^c , KU-2025 ^a , KU-2027 ^a , KU-2028 ^a , KU-2032 ^a , KU-2035 ^a , KU-2039 ^c , KU-2042 ^a , KU-2044 ^c , KU-2050 ^c , KU-2051 ^c , KU-2056 ^c , KU-2058 ^a , KU-2059 ^c , KU-2063 ^c , KU-2066 ^c , KU-2612 ^c , KU-2616 ^c , KU-2617 ^a , KU-2619 ^c , KU-2621 ^c , KU-2624 ^c , KU-2627 ^c , KU-2630 ^a , KU-2632 ^c , KU-2633 ^a , KU-2635 ^c , KU-2636 ^c , KU-2638 ^c , KU-2639 ^c , PI476874 ^c
Armenia (n=19)
IG126273 ^a , IG126280 ^a , IG126293 ^a , IG126353 ^a , IG126991 ^b , IG127015 ^a , IG47173 ^a , IG48747 ^a , IG48748 ^a , IG48758 ^a , KU-2810 ^a , KU-2811 ^a , KU-2814 ^a , KU-2816 ^a , KU-2821 ^a , KU-2822A ^a , KU-2823 ^a , KU-2824 ^a , CGN10734 ^a
Azerbaijan (n=16)
IG47182 ^a , IG47186 ^a , IG47188 ^a , IG47192 ^a , IG47193 ^b , IG47194 ^a , IG47196 ^a , IG47199 ^a , IG47202 ^a , IG47203 ^a , IG47204 ^a , KU-2801 ^a , KU-2804 ^a , KU-2806 ^b , CGN10731 ^a , CGN10732 ^a
China (n=8)
AT47 ^c , AT55 ^c , AT60 ^c , AT76 ^c , AT80 ^c , PI499262 ^a , PI508262 ^a , PI508264 ^c
Dagestan (n=4)
IG120863 ^a , IG120866 ^b , IG48274 ^a , KU-20-1 ^a
Georgia (n=13)
AE1037 ^a , AE454 ^d , AE457 ^d , AE929 ^d , AE933 ^a , KU-2826 ^a , KU-2827 ^b , KU-2828 ^a , KU-2829A ^d , KU-2832 ^d , KU-2834 ^a , KU-2835B ^b , KU-2836 ^a
India (n=1)
IG48042 ^a
Iran (n=64)
IG49095 ^a , KU-2068 ^c , KU-2069 ^a , KU-20-7 ^a , KU-2074 ^{a*} , KU-2075 ^{a*} , KU-2076 ^{b*} , KU-2077 ^{a*} , KU-2079 ^{b*} , KU-2080 ^{b*} , KU-20-8 ^a , KU-20-9 ^{b*} , KU-20-10 ^b , KU-2082 ^c , KU-2083 ^a , KU-2086 ^a , KU-2087 ^c , KU-2088 ^{b*} , KU-2090 ^{b*} , KU-2091 ^{b*} , KU-2092 ^{b*} , KU-2093 ^{a*} , KU2096 ^b , KU-2097 ^a , KU-2098 ^b , KU-2100 ^b , KU-2101 ^b , KU-2102 ^b , KU-2103 ^b , KU-2104 ^a , KU-2105 ^b , KU-2106 ^b , KU-2107 ^b , KU-2108 ^b , KU-2109 ^a , KU-2110 ^a , KU-2111 ^a , KU-2112 ^a , KU-2113 ^a , KU-2115 ^a , KU-2116 ^a , KU-2118 ^a , KU-2120 ^a , KU-2121 ^a , KU-2122 ^a , KU-2124 ^a , KU-2126 ^a , KU-2142 ^a , KU-2143 ^a , KU-2144 ^a , KU-2145 ^a , KU-2148 ^a , KU-2149 ^a , KU-2150 ^a , KU-2151 ^a , KU-2152 ^a , KU-2153 ^a , KU-2154 ^c , KU-2155 ^a , KU-2156 ^a , KU-2157 ^a , KU-2158 ^a , KU-2159 ^a , KU-2160 ^b
Kazakhstan (n=1)
AE1090 ^c
Kyrgyzstan (n=1)
IG131606 ^c
Pakistan (n=14)
IG108561 ^c , IG46666 ^c , IG46666 ^c , IG46682 ^c , KU-2001 ^c , KU-2003 ^c , KU-2006 ^c , KU-2008 ^c , KU-20-6 ^c , CGN10767 ^a , CGN10768 ^c , CGN10769 ^c , CGN10770 ^c , CGN10771 ^c
Syria (n=1)
IG47259 ^a
Tajikistan (n=4)
AE1038 ^a , IG48554 ^a , IG48559 ^c , IG48564 ^a
Turkey (n=13)
KU-2131 ^a , KU-2132 ^a , KU-2133 ^a , KU-2136 ^a , KU-2137 ^a , KU-2138 ^a , KU-2140 ^a , KU-2141 ^a , PI486267 ^a , PI486270 ^a , PI486274 ^a , PI486277 ^a , PI554319 ^a
Turkmenistan (n=6)
IG120735 ^c , IG126387 ^c , IG126489 ^a , IG48508 ^a , IG48518 ^c , CGN10733 ^c
Uzbekistan (n=5)
IG120736 ^c , IG123910 ^c , IG48539 ^c , IG48565 ^c , IG48567 ^c

KU: Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, Japan. PI: National Small Grains Research Facility, USDA-ARS, USA. IG: International Center for Agricultural Research in the Dry Areas (ICARDA). CGN: Centre for Genetic Resources, The Netherlands. AE: Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK). AT: Kenji Kato, Okayama University.

^a HG7 lineage (n=117)

^b HG9 lineage (n=26)

^c HG16 lineage (n=57)

^d HG17 lineage (n=5)

* ssp. *strangulata* (n=12)

floret widths. Spikelet number (NSp) was grouped with spike length (SL). Culm and internode lengths were well correlated with each other, but separated from the spike, spikelet and floret traits. Awn length traits were nearly independent of other traits.

PC analysis of the morphological trait variations

To study intraspecific differentiation in *Ae. tauschii*, we conducted PC analysis based on the 27 morphological traits. Scatter plots with the first two PC values (PC1 and PC2) of the 205 accessions were continuous (Fig. 3A). The accumulated proportion of PC1 and PC2 in the total morphological

Fig. 1. Geographical distribution of *Ae. tauschii* accessions. Adventive populations in the Shaanxi and Henan provinces of China are not shown. Circles, crosses, triangles, and stars indicate the HG7, HG9, HG16, and HG17 lineages, respectively.

Table 2. Variation in 27 morphological traits in 203 accessions

Trait ^a		Total accessions			Subspecies <i>tauschii</i>	Subspecies <i>strangulata</i>
		Mean±standard deviation (SD)	Maximum value	Minimum value	Mean±SD	Mean±SD
SL***	Spike length (cm)	11.12±1.49	6.87	14.92	11.26±1.43	8.99±0.62
NSp**	Number of spikelets per spike	13.31±2.00	8.33	18.33	13.42±2.00	11.56±0.92
TAL	Top awn length (cm)	3.20±0.58	1.32	4.79	3.19±0.59	3.40±0.35
SpL ^b	Spikelet length (mm)	9.42±1.04	7.12	12.23	9.42±1.06	9.36±0.54
GL ^{b***}	Glume length (mm)	6.67±0.50	5.13	8.15	6.71±0.48	6.04±0.46
GW ^{b***}	Glume width (mm)	3.38±0.56	2.28	5.33	3.29±0.43	4.82±0.26
GT ^{b***}	Glume thickness (mm)	0.24±0.03	0.18	0.34	0.23±0.02	0.31±0.02
GH ^{b***}	Glume height (mm)	1.26±0.24	0.88	1.96	1.23±0.21	1.76±0.11
AwL***	Awn length (mm)	5.50±6.17	0	22.53	5.08±5.90	12.27±6.58
LL ^{b**}	Lemma length (mm)	6.91±0.63	5.62	8.72	6.94±0.63	6.49±0.40
LW ^{b***}	Lemma width (mm)	2.91±0.37	2.10	4.02	2.87±0.34	3.59±0.20
AnL***	Anther length (mm)	2.16±0.38	1.49	3.48	2.14±0.38	2.52±0.15
AnW	Anther width (mm)	0.61±0.07	0.43	0.84	0.61±0.07	0.64±0.04
AnT***	Anther thickness (mm)	0.39±0.05	0.23	0.51	0.38±0.05	0.44±0.03
PiL*	Pistil length (mm)	1.37±0.17	0.97	1.93	1.36±0.17	1.47±0.08
PiW**	Pistil width (mm)	1.39±0.18	0.98	1.83	1.38±0.18	1.55±0.09
PiT***	Pistil thickness (mm)	0.92±0.11	0.67	1.48	0.91±0.11	1.05±0.14
PaL ^{b*}	Palea length (mm)	6.71±0.65	5.48	8.68	6.72±0.66	6.44±0.41
PaW ^{b***}	Palea width (mm)	2.56±0.33	1.87	3.58	2.52±0.29	3.20±0.18
AwL4	Awn length of the 4th floret (mm)	4.59±4.14	0	19.28	4.56±4.20	5.06±3.01
ILSp	Internode length between spikelets (mm)	8.70±0.87	7.05	12.28	8.72±0.89	8.47±0.57
MCL***	Main culm length (cm)	68.65±13.02	42.01	111.98	67.71±12.69	83.65±8.32
MCT*	Main culm thickness (mm)	1.60±0.19	1.10	2.10	1.57±0.19	1.70±0.11
IL1	1st internode length (cm)	25.65±5.96	11.58	42.10	25.47±6.04	28.59±3.28
IL2	2nd internode length (cm)	14.42±3.66	8.11	26.52	14.40±3.71	14.76±2.70
IL3	3rd internode length (cm)	10.46±2.04	6.37	20.12	10.50±2.07	9.90±1.36
IL4*	4th internode length (cm)	8.85±1.88	5.20	16.07	8.77±1.86	10.08±1.85

^a Traits that show significant mean difference between the subspecies are indicated by * $P<0.05$, ** $P<0.01$, or *** $P<0.001$ (Student's t test)
^b Matsuoka *et al.* (2009)
 $N=191$ in subspecies *tauschii*, $N=12$ in subspecies *strangulata*

Fig. 2. Relationship of the 27 morphological traits. (A) The relation based on the Pearson coefficient values. Higher values are connected with thicker lines. (B) A dendrogram constructed by the UPGMA method based on the Pearson coefficients. The Pearson values (r) were transformed to the distance values (d) as follows: $d = (1 - r) * 100$.

variation was 50.2% (36.6% for PC1 and 13.6% for PC2). Next, PC analysis was done for spike and spikelet morphology-related traits. A total of 12 traits (SL, NSp, SpL, GL, GW, GT, GH, LL, LW, PaL, PaW and ILSp) were used as spike and spikelet morphology-related traits. The accumulated proportion of PC1 and PC2 in spike and spikelet morphological variation was 74.1% (53.6% for PC1 and 20.5% for PC2). For the spike and spikelet morphology-related traits, the two subspecies formed separate clusters, and scatter plots showed subgroup formation (Fig. 3B). The subspecies *stragulata* cluster consisted of 12 accessions that were plotted with a positive value for PC1 and a value less than -2 for PC2 and did not overlap with the subspecies *tauschii* cluster. On the other hand, plant height-related traits such as SL, NSp, ILSp, MCL, MCT, IL1, IL2, IL3 and IL4 showed a widely distributed pattern in the scatter plots (Fig. 3C). The accumulated proportion of PC1 and PC2 in plant height-related trait variation was 66.9% (42.0% for PC1 and 24.9% for PC2). Subgroup formation was not found in PC analysis based on plant height-related traits.

Geographical clines of morphological traits based on multiple regression analysis

The morphological trait data set provided the opportunity to analyze the diversity patterns of *Ae. tauschii* morphology across its native range. We categorized the examined traits into four groups: plant height-related traits, spike-related traits, anther-related traits, and pistil-related traits. Plant height-related traits included MCL, IL1, IL2, IL3 and IL4. Spike-related traits included SL, NSp, ILSp and SpL. Anther- and pistil-related traits were based on their length, width and thickness. For evaluation of intraspecific variation, PC analysis was done for each trait group, and PC1 and PC2 values were calculated for each accession (Table 3).

Four accessions (CGN 10731, CGN 10732, CGN 10733 and CGN 10734) for which locality data were not available and six accessions (AT 47, AT 55, AT 60, AT 76, AT 80 and PI508264) that represented adventive populations were excluded from this analysis. As a result, PC1 and PC2 of plant height-related traits were found to capture 84.5% of the total variation (69.92% for PC1 and 14.58% for PC2). PC1 and PC2 of spike-related traits captured 76.93% of the variation (52.64% for PC1 and 24.29% for PC2). For anther- and pistil-related traits, 89.14% and 94.28% of the variation were respectively captured by PC1 (75.92% for anther-related traits and 82.08% for pistil-related traits) and PC2 (13.22% for anther-related traits and 12.20% for pistil-related traits). The PC1 and PC2 values were used for comparison between the geographical origins of the *Ae. tauschii* accessions and their morphological traits.

Previous studies reported that multiple regression analysis showed significant effects of latitude and longitude on PC1 values for spikelet-shape variation (Matsuoka *et al.* 2008, 2009). In this study, the diversity patterns of the four groups of morphological traits across the native range were analyzed by multiple regression analysis using the PC1 and PC2 values. In this analysis, the geographical coordinates of the sample collection sites (latitude and longitude) were used as the independent variables. On the basis of the eigenvectors, the PC1 and PC2 values of plant height-related traits and of anther- and pistil-related traits could be used as indices for size and form, respectively (Table 3). The PC1 value for spike-related traits could be used as an index for spike size and spikelet density, whereas the PC2 value could be used for spike size and spikelet size. PC1 values for plant height-related traits, spike-related traits, and anther-related traits were significantly influenced by longitude (Table 4 and Fig. 4). Additionally, the PC2 value for anther-related

Fig. 3. Scatter plots based on the results of principal component (PC) analysis of *Ae. tauschii* morphological variation. White and black circles indicate subspecies *strangulata* and *tauschii* accessions, respectively. (A) PC values for all examined morphological traits. (B) PC values for twelve spike shape-related traits, SL, NSp, SpL, GL, GW, GT, GH, LL, LW, PaL, PaW and ILSp. (C) PC values for nine plant height-related traits, SL, NSp, ILSp, MCL, MCT, IL1, IL2, IL3 and IL4.

traits was significantly related to longitude. A significant effect of latitude on PC1 values of pistil-related traits was also observed.

Table 3. Eigenvectors for PC1 and PC2

Trait	PC1	PC2
Plant height		
MCL	0.51	−0.16
IL1	0.41	−0.70
IL2	0.46	−0.08
IL3	0.42	0.58
IL4	0.42	0.38
Spike shape		
SL	0.56	0.47
NSp	0.67	0.15
ILSp	−0.43	0.45
SpL	−0.23	0.74
Anther shape		
AnL	0.57	−0.68
AnW	0.57	0.73
AnT	0.59	−0.04
Pistil shape		
PiL	0.57	−0.63
PiW	0.60	−0.11
PiT	0.56	0.77

Relationship between morphological variations and chloroplast genome differentiation

The genealogical structure of the morphological variations was examined using 195 *Ae. tauschii* accessions. In our previous studies, four HG lineages, the HG7, HG9, HG16 and HG17 lineages, were defined based on chloroplast DNA differentiation (Matsuoka *et al.* 2008, 2009), and so these 195 accessions of *Ae. tauschii* were divided into the four HG lineages (Table 1 and Fig. 1). Accessions in the HG7 lineage were distributed across the entire range of natural habitats, and the eastward dispersal of *Ae. tauschii* was driven by the HG7 and HG16 lineages (Matsuoka *et al.* 2008). The HG9 lineage accessions were specific to western habitats, and the distribution area of five HG17 accessions was only found in Georgia.

Significant differences were found for some pairs of mean PC values (Table 5). The HG16 lineage exhibited a smaller mean than HG7 for PC2 values of plant height variation. For PC1 values of spike-shape variation, the accessions of the HG9 lineages had a significantly smaller mean than the accessions of the HG7 and HG16 lineages. Similarly, means of the HG16 lineage were significantly different from those of HG7 and HG9 lineages for the PC1 and PC2 values of anther-shape variation. Furthermore, there was a significant difference in the PC1 values of pistil-shape variation between the HG7 and HG9 lineages. No significant difference in the means was observed for the PC1 values of plant height variation and the PC2 values of spike-shape and pistil-shape variations.

Discussion

Natural morphological variation in Ae. tauschii

Iran is a center of genetic variation of *Ae. tauschii*

Table 4. Multiple regression analysis of the effects of latitude and longitude on morphological traits using 195 accessions

Trait	Principal component	Source	Parameter estimate	Standard error	<i>t</i> statistic	<i>P</i>
Plant height	first	latitude	-0.015	0.06	-0.30	0.7637
	first	longitude**	-0.043	0.02	-3.05	0.0026
	second	latitude	0.024	0.023	1.04	0.2979
	second	longitude	-0.007	0.007	-1.01	0.3146
Spike shape	first	latitude	-0.002	0.037	-0.04	0.9662
	first	longitude***	0.043	0.011	3.98	<0.0001
	second	latitude	0.020	0.026	0.74	0.4597
	second	longitude	-0.001	0.008	-0.16	0.8719
Anther shape	first	latitude	-0.070	0.040	-1.78	0.0772
	first	longitude***	-0.045	0.011	-3.94	0.0001
	second	latitude	0.001	0.016	0.04	0.9686
	second	longitude***	0.021	0.005	4.44	<0.0001
Pistil shape	first	latitude***	-0.160	0.041	-3.91	0.0001
	first	longitude	0.001	0.012	0.11	0.9119
	second	latitude	0.022	0.016	1.35	0.1791
	second	longitude	-0.003	0.005	-0.66	0.5086

This analysis was based on a bi-variate linear regression model using latitudinal and longitudinal locations (degrees) as independent variables. Significant effects are indicated by asterisks.

Fig. 4. Geographical clines of morphological trait variations ($N=195$). (A) The relationship between longitude of origin and plant height. (B) The relationship between longitude and spike shape. (C) The relationship between longitude and anther shape. (D) The relationship between latitude of origin and pistil shape.

Table 5. Comparison of mean principal component values of the four intraspecific lineages

Trait	Principal component	Lineage	N	Mean	Standard error
Plant height	first	HG7	114	0.25 ^a	0.19
		HG9	26	-0.01 ^a	0.35
		HG16	50	-0.49 ^a	0.20
		HG17	5	-0.72 ^a	0.45
	second	HG7	114	0.14 ^{ab}	0.08
		HG9	26	-0.26 ^{bc}	0.14
		HG16	50	-0.26 ^c	0.10
		HG17	5	0.81 ^a	0.39
Spike shape	first	HG7	114	0.05 ^b	0.13
		HG9	26	-1.90 ^c	0.20
		HG16	50	0.85 ^a	0.12
		HG17	5	0.21 ^{ab}	0.42
	second	HG7	114	-0.01 ^a	0.09
		HG9	26	0.10 ^a	0.27
		HG16	50	-0.02 ^a	0.11
		HG17	5	-0.02 ^a	0.78
Anther shape	first	HG7	114	-0.12 ^b	0.15
		HG9	26	1.54 ^b	0.22
		HG16	50	-0.62 ^a	0.11
		HG17	5	0.94 ^{ab}	0.33
	second	HG7	114	-0.06 ^b	0.06
		HG9	26	-0.27 ^b	0.12
		HG16	50	0.32 ^a	0.07
		HG17	5	-0.37 ^{ab}	0.10
Pistil shape	first	HG7	114	-0.31 ^b	0.16
		HG9	26	0.76 ^a	0.26
		HG16	50	0.32 ^{ab}	0.19
		HG17	5	-0.14 ^{ab}	0.49
	second	HG7	114	0.06 ^a	0.06
		HG9	26	-0.13 ^a	0.10
		HG16	50	-0.09 ^a	0.08
		HG17	5	0.35 ^a	0.16

For each principal component, mean values followed by the same letters were not significantly different ($P > 0.05$) (Tukey-Kramer's HSD test).

(Dudnikov and Goncharov 1993). In this study, *Ae. tauschii* exhibited large natural variations in the examined morphological traits. Scatter plots of PC analysis based on these morphological traits showed high levels of variation in each habitat from the western region to China. This finding indicated that the *Ae. tauschii* accessions in the non-Iranian habitats carried a large degree of genetic variation in morphological traits. The birthplace of common wheat has been assumed to be Transcaucasia or the south coastal region of the Caspian Sea (Tsunewaki 1966, Jaaska 1980, Nishikawa *et al.* 1980, Dvorak *et al.* 1998). Common wheat was derived from single or limited accessions of *Ae. tauschii*, and *Ae. tauschii* populations far from its birthplace were not involved in the formation of common wheat.

In this study, all examined traits except awn length-

related traits showed continuous distribution. The awn length-related traits were correlated with each other, but independent of other morphological traits. These results suggested that most of the examined morphological traits may be determined by quantitative trait loci (QTL), whereas the awn length traits might be genetically controlled by a small number of major genes. There is little information about chromosomal location of the genetic loci generating the morphological diversity of *Ae. tauschii*. Detection of the QTLs and major genes that control these examined traits are required to understand the genetic framework for morphological diversification in *Ae. tauschii*.

Recognition of subspecies division in *Ae. tauschii* is controversial, because morphologically and genetically intermediate forms exist (Van Slageren 1994). In a previous study, we applied *sensu stricto* criteria for subspecies *strangulata* identification, defining it as having markedly moniliform spikes (Matsuoka *et al.* 2009). Our numerical analysis of spikelet morphology clearly distinguished the subspecies *strangulata* accessions from subspecies *tauschii* accessions. A similar result was obtained based on the natural genetic variation in the 12 traits SL, NSp, SpL, GL, GW, GT, GH, LL, LW, PaL, PaW and ILSp (Fig. 3B), although no clear subspecies division was observed in PC analyses using all the examined traits and plant height-related traits. Thus, the two *Ae. tauschii* subspecies, *tauschii* and *strangulata*, could be distinguished based on the PC1 and PC2 values of spike-related morphological variation. The individual traits of spike and spikelet are in fact continuous in the *Ae. tauschii* populations (Van Slageren 1994, Dudnikov 2000), which resulted in questioning the biological significance of the subspecies division. Our results showed patterns of intraspecific morphological differentiation in the *Ae. tauschii* populations. As previously reported, GW is an important morphological trait to consider for the intraspecific differentiation of the two *Ae. tauschii* subspecies (Eig 1929, Dudnikov 2000, Dudnikov and Kawahara 2006). Our study also showed the importance of spikelet and floret shape-related traits including GW. GW was highly correlated with GH, LW and PaW (Fig. 2A), meaning that the same genes might control these traits. Further mapping of QTL controlling spikelet and floret morphology-related traits and subsequent gene isolation for the detected QTLs are required to address the molecular nature of differentiation in subspecies *strangulata*.

Geographical clines of morphological variation

The putative primary region of *Ae. tauschii*'s origin is the Transcaucasus (Van Slageren 1994). From the Transcaucasus, the distribution area of *Ae. tauschii* expanded through eastward and southward dispersal. In this study, significant geographical clines were detected for each of the four morphological trait groups: plant height-related traits, spike-related traits, anther-related traits and pistil-related traits (Table 4 and Fig. 4). Our results indicated that (1) culm length tends to be short in the eastern region of the native range, (2) the spike tends to be long in the eastern

region, (3) anthers tend to be small in the eastern region, and (4) pistils tend to be small in the northern region. Furthermore, in the eastern habitats, spikelet density per spike tended to be high, because eigenvectors of ILSp and SpL were negative for PC1 values of spike shape variation, in contrast to those of SL and NSp (Table 3).

Our previous study showed that the *Ae. tauschii* populations differentiated to the four chloroplast haplogroup lineages (Matsuoka *et al.* 2008). The largest HG7 lineage distributed across the current species' natural distribution range, whereas the HG9 and HG16 haplogroup lineages were confined to either the eastern or western habitats. The distribution area of five HG17 accessions was limited in Georgia. PC1 and PC2 values from the spike and anther shape-related traits in HG16 were significantly different from those in HG9, and significant difference of PC2 from the plant height-related traits was observed between HG16 and HG17 (Table 5). Therefore, the geographical clines for the morphological variations were consistent with results from the genealogical analysis using chloroplast DNA differentiation patterns.

The latitudinal cline found for the pistil size deserves a note. Matsuoka *et al.* (2009) analyzed spikelet-shape variation of 203 *Ae. tauschii* accessions and indicated that the spikelet size tends to be small in the southern region of the native range. Thus, the orientation of the pistil-size cline (small in the north, large in the south) appeared to be different from that of the spikelet-size cline (large in the north, small in the south). Reevaluation of the Matsuoka *et al.* (2009)'s work, however, identified an error that had been made in interpreting the data from multiple regression analysis. In that analysis, a negative correlation between the PC1 values for spikelet-shape variation and latitude was found. The correct interpretation of the data was that the spikelet size tends to be small in the northern region of the native range, because the PC1 values changed from low to high as spikelet size changed from small to large. Accordingly, Matsuoka *et al.* (2009)'s remark on the orientation of the latitudinal spikelet-size cline is wrong and needs to be corrected. When this correction is made, the orientation of the pistil-size and spikelet-size clines is the same: the accessions that are derived from northern regions of the native range tend to have small pistils and spikelets.

The longitudinal geographic clines found for several vegetative and reproductive organs suggest that, in the process of eastward dispersal from Transcaucasus, *Ae. tauschii* underwent morphological, genetic, and ecological diversification that produced the variation seen among today's natural populations. Previously, we reported the existence of significant longitudinal clines in variation of flowering time, which is a reproductive trait having a significant impact on fitness, implying that an early-flowering phenotype played an important role for *Ae. tauschii* to adapt to the eastern regions of its native range (Matsuoka *et al.* 2008). Similarly, small spikelets, short culms, long spikes with high spikelet density, and small anthers might reflect some type of

adaptation to local conditions of the eastern habitats. The mechanisms underlying the morphological changes remain to be addressed, whereas relatively short vegetative and reproductive growth periods due to continental climate of the east might be a factor that affected the clinal patterns of morphological diversification. Determination of the genetic locus controlling such morphological changes may help understand the genetic mechanisms that underlie the adaptation to local conditions of the eastern habitats.

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