



Allozyme Variation in Turkmenian Populations of Wild Barley, *Hordeum spontaneum* Koch.

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The extent and structure of genetic variation in 720 individuals representing 36 populations of wild barley, *Hordeum spontaneum*, from Central Asia (Turkmenistan) were determined using starch gel electrophoresis of eight water soluble leaf proteins encoded by 13 loci. The populations were grouped into seven ecogeographic regions. The study found: (a) a similar amount of within population genetic diversity ($H_e = 0.106$), but lower total genetic diversity ($H_T = 0.166$) to that reported for Middle East populations of *H. spontaneum*; (b) of the total genetic diversity, 61 % was attributable to variation within populations, 27 % between populations of a region, and 12 % among regions; (c) of the 42 alleles found, 11 were ubiquitous, 22 were widespread and common, three local and common and seven local and rare; (d) there was a poor correlation between population genetic and geographic distances; and (e) the frequencies of only a few alleles correlated significantly with climatic or geographic parameters. The extent and structure of genetic variation of Turkmenian populations, which represent the Central Asian part of the species' range, were significantly different in some important aspects from Middle Eastern and eastern Mediterranean populations. The mosaic pattern of genetic variation found in wild barley in the Middle East is less pronounced in populations from Central Asia where there is less genetic differentiation among populations and regions, and more ubiquitous or common and fewer localized alleles.

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INTRODUCTION

Wild barley, *Hordeum spontaneum* Koch., is the progenitor of cultivated barley, *H. vulgare* L. (Harlan and Zohary, 1966; Zohary, 1969; Harlan, 1976). *H. spontaneum* is a common species in the Middle East and the eastern Mediterranean, as well as in southern Central Asia and Tibet. It is an almost exclusively autogamous annual grass whose spikes have a brittle rachis and contain two rows of spikelets. Seed dispersal is usually limited to within several metres of the mother plant, although seeds can be carried in the fur of animals over longer distances (Zohary, 1969). *H. spontaneum* is often a predominant annual component of open parks and herbaceous formations, but can also be found along roadsides, abandoned fields and at the edges of cultivated fields (Harlan and Zohary, 1966; Zohary, 1973). *H. spontaneum* is found from sea level up to an altitude of 1500 m and in both xeric and mesic environments (between 100–1500 mm annual rainfall). Both *H. spontaneum* and *H. vulgare* can easily be crossed, and agronomically important genes have been introgressed into *H. vulgare*

from *H. spontaneum* (Lehmann, 1991; Anikster *et al.*, 1992; Fischbeck and Jahoor, 1992). Natural hybrids can occur when the two species coexist in the same field.

The two regions in which *H. spontaneum* is found, i.e. the Middle East (including the eastern Mediterranean) and Central Asia (including Tibet), have very different climates. The former primarily has a Mediterranean climate with short, wet, mild winters and long, hot summers, whereas Central Asia has a continental or mountain climate with cold, long winters, relatively short summers and a much greater difference between winter and summer temperatures. While *H. vulgare* is thought by most researchers to have been domesticated from *H. spontaneum* about 10 000 years ago in the Fertile Crescent (reviewed by Harlan, 1992), evidence has recently been presented to indicate its possible polyphyletic origin (Xu, 1982; Ma *et al.*, 1987; Molina-Cano *et al.*, 1987, 1999; Moralejo *et al.*, 1994). It was hypothesized that domestication of *H. spontaneum* evolved independently in the Oriental region (Xu, 1982; Zhang *et al.*, 1994).

Over the past 20 years, an extensive amount of information has been obtained on the extent and structure of genetic variation in *H. spontaneum* from environmentally milder Southwest Asia (Nevo *et al.*, 1979, 1986a, c; Moseman *et al.*, 1980; Saghai Moroofof *et al.*, 1984, 1990;

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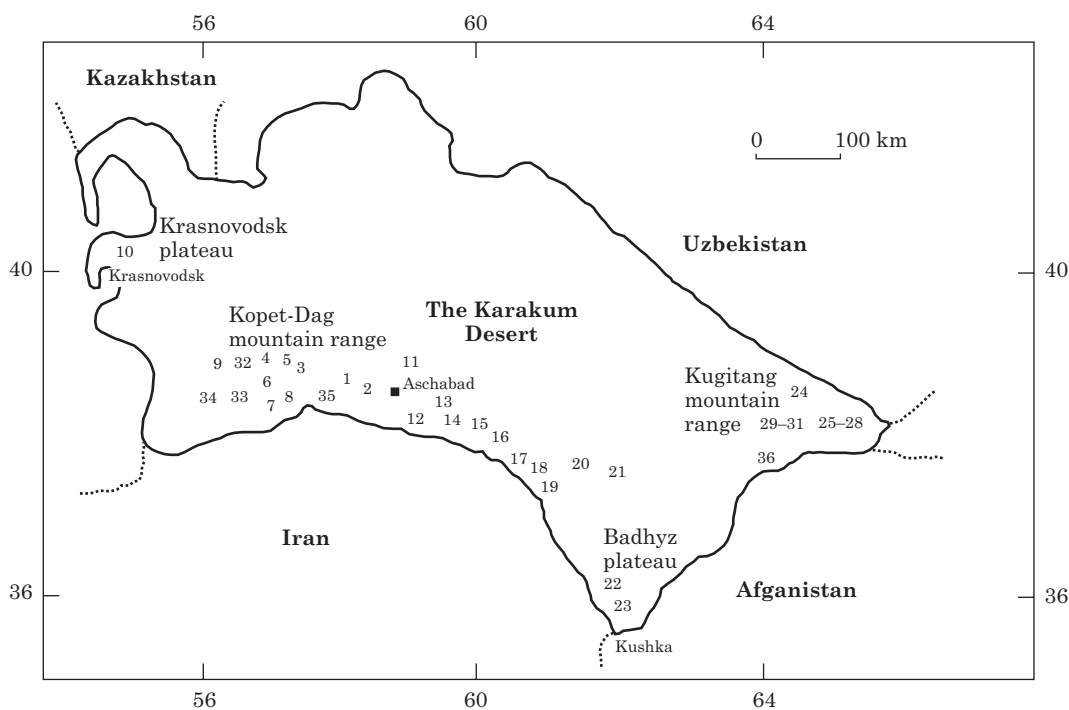


FIG. 1. Geographic distribution of the sampled populations of *H. spontaneum* from Turkmenistan.

Snow and Brody, 1984; Jana and Pietrzak, 1988; Neale *et al.*, 1988; Zhang *et al.*, 1993). Little comparable genetic information is available for populations from the northern or eastern parts of *H. spontaneum*'s distribution; i.e. from the environmentally harsher Central Asia. This lack of information on genetic variation in wild barley populations across approximately half of its range has prevented a complete understanding of the extent and structure of genetic variation in *H. spontaneum*, its relationship to ecological and environmental factors, and the phylogeny and evolution of cultivated barley.

Within a larger study comparing the extent and structure of genetic variation in *Hordeum spontaneum* populations originating from the Middle East vs. Central Asia, this paper presents the results of an electrophoretic analysis of 13 water soluble leaf proteins in 36 populations collected from Central Asia, specifically from Turkmenistan. Starch gel electrophoresis of leaf proteins was chosen because this method was used in most of the previous studies from the Middle East and therefore allows a comparison between our results and those previously published. Morphological and RAPD variation will be reported in subsequent papers.

MATERIALS AND METHODS

Population sampling

Fifty spikes, each from a separate plant, were collected from each of 36 Turkmenian populations during two expeditions to Turkmenistan (May 1992 and 1993). The sampled populations cover the range of environments occupied by *H. spontaneum* in Turkmenistan. The location and geographical, meteorological and ecological data on each

population are given in Tables 1 and 2 and their distribution is shown in Fig. 1. Based on geomorphological and vegetative data, the populations were divided into six ecogeographic regions: (1) Kopet-Dag mountains (13 populations); (2) Kopet-Dag foothills (8); (3) Karakum desert (3); (4) Kugitang mountains (4); (5) Kugitang foothills (5); (6) Badkhyz plateau (2) (Table 1). In addition, one population was collected from the Krasnovodsk plateau. Population size was estimated and each population was characterized as small (<1000 plants) or large (>1000 plants). The small number of populations collected in regions other than the Kopet-Dag mountains and foothills was a result of either their environmental unfavourability (Karakum Desert and Krasnovodsk plateau) or because of their relatively small area (Badkhyz plateau, Kugitang mountains and foothills).

The collection protocols in each population followed Marshall and Brown (1975) as modified by Mendlinger and Zohary (1995). A collection was made along one or two linear transects covering as much of a population's area as possible. In each population, plants at least 2 m apart were sampled by collecting one spike from each plant. Each spike was placed in a separate bag.

Electrophoresis

We used horizontal starch gel electrophoresis to examine allozyme variation in eight enzyme systems encoded by 13 loci. The protocol and recipes followed Brown *et al.* (1978), Brody and Mendlinger (1980) and Mendlinger and Zohary (1995). One seed from each selected spike was germinated and grown in a greenhouse. Leaves from 2-week-old seedlings were cut, macerated in two drops of distilled

TABLE 1. Ecogeographical data for 36 populations of *H. spontaneum* from Turkmenistan

No.	Location	Geomorphology	Ecology		
			Soil type	Habitat	Pop. size
1	Seliysky	Kopet-Dag mountain range	Mountain grey soil	Defile	Large
2	Chuli (2 km NW)	Kopet-Dag mountain range	Mountain grey soil	South slope	Large
3	Beurme (7 km S)	Kopet-Dag mountain range	Mountain grey soil	East slope	Large
4	Bami (10 km SW)	Kopet-Dag mountain range	Mountain grey soil	Wadi	Large
5	Bami (12 km SW)	Kopet-Dag mountain range	Mountain grey soil	Slope	Large
6	Kara-Kala (15 WNW)	Kopet-Dag mountain range	Mountain grey soil	Defile	Small
7	Kara-Kala (7 km SSE)	Kopet-Dag mountain range	Mountain grey soil	Wadi	Small
8	Kara-Kala (20 km SSE)	Kopet-Dag mountain range	Mountain grey soil	Mountain pass	Large
9	Iskander (60 km SW)	Kopet-Dag mountain range	Mountain grey soil	Wadi	Large
10	Krasnovodsk (1 km W)	Krasnovodsk plateau	Basalt	Shallow wadi	Small
11	Aschabad (10 km NW)	Karakum Desert	Sand	Roadside	Large
12	Aschabad (15 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Roadside	Large
13	Aschabad (20 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Roadside	Large
14	Aschabad (30 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Roadside	Large
15	Aschabad (35 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Roadside	Large
16	Aschabad (50 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Wadi	Small
17	Aschabad (60 km SE)	Kopet-Dag foothill plain	Grey brown desert soil	Wadi	Large
18	Kaahna (2 km NW)	Kopet-Dag foothill plain	Grey brown desert soil	Plain and wadi	Large
19	Dushak (10 km W)	Kopet-Dag foothill plain	Grey brown desert soil	Shallow wadi	Large
20	Serachs (40 km NNW)	Karakum Desert	Sand	Ravine	Large
21	Serachs (3 km SSE)	Karakum Desert	Takyr	Abandoned field	Large
22	Kepelya (20 km NW)	Badhyz plateau	Grey soil	Plain	Large
23	Kepelya (1 km S)	Badhyz plateau	Mountain grey soil	East slope	Large
24	Karluk (5 km W)	Kugitang foothill plain	Clay	Wadi	Large
25	Karluk (10 km N)	Kugitang mountain range	Clay	Wadi	Large
26	Karluk (10 km N)	Kugitang mountain range	Clay	Defile	Large
27	Karluk (15 km N)	Kugitang mountain range	Mountain grey soil	Few ravines	Small
28	Karluk (10 km E)	Kugitang mountain range	Mountain grey soil	Defile	Large
29	Charshanga (17 km NE)	Kugitang foothill plain	Clay (salty)	Plain	Large
30	Gurdak (10 km SE)	Kugitang foothill plain	—	Wadi	Large
31	Gurdak (3 km SE)	Kugitang foothill plain	—	Shallow defile	Large
32	Kara-Kala (25 km NW)	Kopet-Dag mountain range	Clay	Wadi	Small
33	Kizil-Arvat (70 km SSW)	Kopet-Dag mountain range	Clay	Wadi	Large
34	Kizil-Arvat (80 km SSW)	Kopet-Dag mountain range	Clay	Wadi	Large
35	Chuli (1 km NW)	Kopet-Dag mountain range	Mountain grey soil	Slope	Large
36	Charshanga (5 km NE)	Kugitang foothill plain	Clay	Wadi	Large

—, no data available.

TABLE 2. Geographical and climatic data for 36 populations of *H. spontaneum* from Turkmenistan (see text for details)

No.	Geography			Climate					
	Longitude (Ln)	Latitude (Lt)	Altitude (Al)	Annual (Tm)	Temperature (Ta)	January (Tj)	Rainfall (Rn)	Humidity (%) (Hu)	Evaporation (cm) (Ev)
1	58-60	37-85	600	15-4	27-8	0-7	383	58	132
2	58-00	38-00	670	14-1	25-2	1-8	268	58	132
3	56-90	38-60	440	14-4	27-4	0-0	270	56	136
4	56-70	38-65	500	14-4	27-4	0-0	295	56	136
5	56-75	38-60	800	14-4	27-4	0-0	295	54	136
6	56-15	38-50	480	16-1	27-6	3-6	320	54	135
7	56-45	38-35	480	15-8	27-7	3-7	404	54	135
8	56-35	38-25	1130	12-9	24-0	1-0	457	54	135
9	55-35	38-80	340	16-0	28-2	3-2	238	56	165
10	52-95	40-00	100	14-2	27-3	1-7	150	61	140
11	58-30	38-00	150	16-1	28-9	0-9	220	54	150
12	58-60	37-90	150	16-1	28-9	0-9	220	54	150
13	58-65	37-85	150	16-1	28-9	0-9	220	54	150
14	58-75	37-80	150	16-1	28-9	0-9	220	54	150
15	58-80	37-80	150	16-1	28-9	0-9	220	54	150
16	58-90	37-75	150	16-1	28-9	0-9	220	54	150
17	59-05	37-60	160	16-1	28-9	0-9	213	54	150
18	59-55	37-40	170	16-3	29-0	1-4	245	52	169
19	59-90	37-20	170	16-3	29-0	1-4	211	52	169
20	61-05	36-85	200	16-7	29-2	1-8	205	49	170
21	61-25	36-50	300	16-6	28-3	3-0	225	47	185
22	61-40	35-95	160	14-5	25-8	2-4	290	48	174
23	61-50	35-80	880	14-5	25-8	2-4	285	48	174
24	66-25	37-60	300	18-0	30-2	3-4	185	45	231
25	66-30	37-65	300	16-3	28-5	2-7	379	45	231
26	66-30	37-65	350	16-3	28-5	2-7	379	45	231
27	66-35	37-70	300	16-3	28-5	2-7	379	45	231
28	66-40	37-60	500	16-3	28-5	2-7	379	45	231
29	66-20	37-60	300	18-0	30-2	3-4	185	45	231
30	66-15	37-75	350	16-3	28-5	2-7	272	45	231
31	66-10	37-80	350	16-3	28-5	2-7	272	45	231
32	56-05	38-55	200	16-1	27-6	3-6	302	54	135
33	55-75	38-35	200	16-1	27-6	3-6	302	54	135
34	55-80	38-30	180	16-1	27-6	3-6	302	54	135
35	58-00	38-00	680	13-5	24-4	1	268	58	132
36	66-10	37-55	300	18-0	30-2	3-4	185	45	231

water and a drop of bromophenol blue, the extract absorbed on filter paper wicks and placed into a gel. The starch gel was prepared by boiling 39 g of hydrolysed potato starch (Sigma, S-4501) dissolved in 300 ml gel buffer with subsequent degassing by a vacuum pump. Three buffer systems, Tris-citrate (TC), Tris-malate (TM) and a Poulik discontinuous buffer (Poulik) were used. Samples from 15 different *H. spontaneum* plants, five each from three populations, plus a control (a commercial *H. vulgare* cultivar) were placed in each gel. Gels were run in a refrigerator at 4°C with a constant current of 100 mA and a voltage of 100 (TM), 110 (TC) or 200 V (Poulik) for 3–4 h. After electrophoresis, each gel was sectioned into three slices, and each slice was stained for a different enzyme. The enzymes examined, the abbreviations employed, E.C. numbers, number of loci in each enzyme system and the buffer used are as follows:

- (1) Catalase, Cat; E.C. 1.11.1.6; one locus. Poulik buffer
- (2) Glutamate dehydrogenase, Gdh; E.C. 1.4.1.2; one locus. TC buffer
- (3) Esterase, Est; E.C. 3.1.1.2; three loci. TC buffer
- (4) Malate dehydrogenase, Mdh; E.C. 1.1.1.37; two loci. TM and TC buffer
- (5) Phosphoglucumutase, Pgm; E.C. 2.7.5.1; one locus. TM buffer
- (6) Phosphoglucose isomerase, Pgi; E.C. 5.3.1.9; two loci. TM buffer
- (7) 6-Phosphogluconate dehydrogenase, 6-Pgd; E.C. 1.1.1.44; two loci. TM buffer
- (8) General protein, Gp; E.C. 4.1.1.39; one locus. TM buffer

After staining the gels were read, fixed in 10 % glacial acetic acid for 24 h and stored at 4°C. Alleles were scored according to their mobility on the gel with 'a' being the fastest.

Statistical analysis

All analyses of genetic data were performed using POPGENE version 1.31 (Yeh *et al.*, 1998). The amount of genetic variation for each population was estimated by calculating the mean number of alleles per locus, n_a ; the effective number of alleles, n_e ; the proportion of polymorphic loci, P ; and the percent of expected (H_e) and observed (H_o) heterozygotes. The advantage of using n_e over n_a is that the effective number of alleles is determined more by the uniformity of allele frequencies than by the actual number of alleles. The same indices, n_a , n_e , P and H_e were calculated from regional gene pools to estimate descriptive statistics for grouped populations. Nei's unbiased measures of genetic distance (Nei, 1978) were calculated and used in a cluster analysis (unweighted pair groups means method, UPGMA). The partitioning of total genetic diversity (H_T) into its three components, within populations (H_C), between populations of a region (G_{CS}) and among regions (G_{ST}), was accomplished using Nei's G_{ST} statistic (Nei, 1973). Stepwise multiple regression was performed to determine the degree of association between environmental factors and allele frequencies

(JMP statistical program). Analysis of variance was used to determine differences among groups (after arcsine transformation of all percentages to meet the requirements for equality of variance and normal distribution) and the G-test to examine Goodness-of-Fit (Sokal and Rohlf, 1981).

RESULTS

Allele frequencies

A total of 42 alleles were found over the 13 loci in the 36 populations (Appendix and Table 3). The distribution pattern of the 42 alleles can be characterized using a modified classification system based on Marshall and Brown (1975) which places alleles into four groups according to their frequency within populations and the extent of their geographic distribution. The four groups are: (1) ubiquitous (found in all populations)—11 alleles, one in each locus except Est-3 and Cat, were ubiquitous and found in high frequency in all populations; (2) widespread—22 alleles were widespread (in at least five populations over at least three regions) with most being moderate to low frequency; (3) localized and common—three alleles, Est-4-d, Pgm-a and Mdh-1-a, were found in fewer than five populations but were usually common in these populations (more than 10 % in most populations); and (4) localized and rare—seven alleles were localized and rare (less than 1 % over all populations) but only one allele, Pgi-2-a, was unique to a population (population 6). When the populations were subdivided into the six regions, only four alleles were found to be unique to a region (Table 4): Est-1-a (only in Kugitang foothills); Est-4-d (only in Kopet-Dag mountains); Pgi-2-a (only in Kopet-Dag mountains); and MDH-2-a (only in Kugitang mountains). Several other alleles were found in neighbouring regions. Est-4-c was found only in the Kopet-Dag and Kugitang mountains. Therefore, the overall pattern is for widespread sharing of alleles among populations and regions, with relatively few unique or localized alleles.

Extent of variation

The extent of genetic variation varied between populations (Table 4). The mean number of alleles per locus, n_a , was 1.50 but ranged between 1.23 and 2.08; the effective number of alleles, n_e , was 1.18 (range 1.05–1.37); the proportion of polymorphic loci per population, P , was 0.41 (range 0.23–0.77); the proportion of heterozygosity per locus per individual, H_o , was 0.001 (range 0.000–0.008); and the genetic diversity, H_e , was 0.095 (range 0.042–0.216). No significant difference was found between regions in either n_a , n_e or H_e , although there was a trend for Kopet-Dag and Kugitang mountains to have higher H_e and P than other populations (Table 5). No significant differences were found among populations from the mountains, foothills and plains, however, n_a , H_e and P seemed to decrease from elevated areas to the plains (Table 5). The very low H_o as compared to H_e supports previous findings that *H. spontaneum* is primarily autogamous.

TABLE 3. Allele frequencies in 13 loci of *H. spontaneum* from six regions in Turkmenistan

Locus/allele	Region					
	Kopet-Dag mountains	Kopet-Dag foothills	Karakum desert	Kugitang mountains	Kugitang foothills	Badhyz plateau
Est-1						
a	—	—	—	—	0.08	—
b	0.99	1.00	1.00	0.58	0.82	0.95
c	0.01	—	—	0.42	0.10	0.05
Est-3						
a	0.09	0.02	—	0.06	0.08	—
b	0.45	0.27	0.48	0.75	0.76	0.55
c	0.26	0.10	0.13	0.19	0.16	0.45
d	0.20	0.61	0.39	—	—	—
Est-4						
a	0.13	0.04	—	0.02	0.10	0.18
b	0.80	0.96	1.00	0.98	0.72	0.82
c	0.03	—	—	—	0.18	—
d	0.04	—	—	—	—	—
Cat						
a	0.02	—	—	0.02	0.02	—
b	0.65	0.86	0.65	0.96	0.85	0.98
c	0.33	0.14	0.35	0.02	0.13	0.02
GP						
a	0.02	0.02	0.08	0.03	0.04	—
b	0.97	0.97	0.92	0.97	0.95	0.97
c	0.01	0.01	—	—	0.01	0.03
Gdh						
a	—	—	—	0.01	0.02	—
b	1.00	1.00	1.00	0.98	0.97	1.00
c	—	—	—	0.01	0.01	—
Pgm						
a	0.02	0.11	—	—	—	—
b	0.98	0.89	1.00	0.99	0.97	1.00
c	—	—	—	0.01	0.03	—
Pgi-1						
a	—	—	0.07	0.04	0.03	—
b	1.00	0.88	0.88	0.95	0.96	0.93
c	—	0.12	0.05	0.01	0.01	0.07
Pgi-2						
a	0.01	—	—	—	—	—
b	0.01	0.01	0.02	—	—	—
c	0.97	0.98	0.98	1.00	1.00	1.00
d	0.01	0.01	—	—	—	—
6-pgd-1						
a	0.04	0.03	—	—	0.15	0.02
b	0.93	0.89	0.92	1.00	0.83	0.93
c	0.03	0.08	0.08	—	0.02	0.05
6-pgd-2						
a	0.03	0.01	0.05	0.04	0.07	—
b	0.95	0.98	0.95	0.96	0.93	0.95
c	0.01	0.01	—	—	—	0.05
Mdh-1						
a	0.03	—	—	0.06	0.05	—
b	0.90	1.00	0.98	0.94	0.88	1.00
c	0.07	—	0.02	—	0.07	—
Mdh-2						
a	—	—	—	0.04	—	—
b	0.30	1.00	1.00	0.91	0.96	1.00
c	0.70	—	—	0.05	0.04	—

TABLE 4. Within-population genetic diversity estimates for Turkmenian populations of wild barley

Population	Mean number of alleles per locus (n_a)	Effective number of alleles (n_e)	Proportion polymorphic loci (P)	Expected heterozygosity (H_e)	Observed heterozygosity (H_o)	Mean genetic distance (D)
1	1.38	1.22	0.31	0.108	0	0.099
2	1.46	1.13	0.46	0.097	0	0.106
3	1.46	1.07	0.38	0.056	0	0.060
4	1.30	1.05	0.23	0.042	0	0.058
5	1.54	1.14	0.54	0.100	0	0.060
6	1.46	1.27	0.38	0.142	0	0.192
7	1.69	1.30	0.46	0.148	0.004	0.071
8	1.46	1.24	0.38	0.139	0	0.121
9	1.31	1.05	0.31	0.042	0	0.083
10	1.23	1.08	0.23	0.053	0	0.144
11	1.46	1.08	0.38	0.062	0.008	0.085
12	1.31	1.15	0.31	0.096	0	0.063
13	1.54	1.11	0.38	0.072	0	0.126
14	1.54	1.15	0.38	0.103	0	0.055
15	1.38	1.08	0.31	0.053	0	0.074
16	1.38	1.20	0.31	0.101	0.004	0.108
17	1.46	1.16	0.38	0.104	0	0.098
18	1.62	1.16	0.46	0.110	0.008	0.065
19	1.31	1.08	0.23	0.057	0	0.053
20	1.38	1.25	0.31	0.119	0	0.058
21	1.38	1.18	0.38	0.116	0	0.092
22	1.38	1.12	0.38	0.084	0	0.098
23	1.46	1.06	0.38	0.050	0	0.075
24	1.69	1.32	0.54	0.185	0	0.070
25	1.92	1.30	0.77	0.201	0	0.062
26	1.23	1.16	0.23	0.090	0	0.073
27	1.38	1.13	0.23	0.077	0	0.087
28	1.46	1.13	0.38	0.088	0	0.061
29	1.46	1.21	0.38	0.122	0.004	0.061
30	1.85	1.23	0.61	0.143	0.008	0.061
31	1.70	1.24	0.62	0.158	0	0.066
32	1.70	1.23	0.46	0.124	0	0.059
33	1.77	1.21	0.54	0.128	0	0.053
34	1.54	1.37	0.31	0.144	0	0.123
35	1.54	1.17	0.46	0.094	0	0.054
36	2.08	1.32	0.77	0.216	0.008	0.053
Mean	1.50	1.18	0.41	0.106	0.001	0.079
s.e.	0.032	0.014	0.023	0.007	0.0004	0.005

$n = 20$.

TABLE 5. Grouped populations' descriptive statistics for two geographical groupings

Grouping	Number of populations	Mean number of alleles per locus (n_a)	Effective number of alleles (n_e)	Expected heterozygosity (H_e)	Proportion polymorphic loci (P)
A. Regions					
Kopet-Dag	13	2.85 (0.80)	1.33 (0.58)	0.169 (0.205)	1.00
Kopet-Dag foothills	8	2.15 (0.99)	1.19 (0.32)	0.123 (0.155)	0.69
Karakum Desert	3	1.77 (0.72)	1.24 (0.44)	0.133 (0.192)	0.61
Kugitang mountains	4	2.23 (0.72)	1.18 (0.29)	0.123 (0.150)	0.85
Kugitang foothills	5	2.61 (0.65)	1.27 (0.25)	0.187 (0.143)	0.92
Badhis plateau	2	1.69 (0.63)	1.16 (0.27)	0.104 (0.144)	0.61
B. Environment					
Mountains	17	3.15 (0.55)	1.29 (0.47)	0.169 (0.180)	1.00
Foothills	13	3.00 (0.41)	1.27 (0.45)	0.164 (0.163)	1.00
Plains	6*	2.15 (0.08)	1.27 (0.51)	0.142 (0.195)	0.76

*Includes population 15 from Krasnovodsk plateau.

Indices are calculated on pooled allele frequencies of populations comprising a group. Means over 13 loci with standard errors (in parentheses) are shown.

TABLE 6. Mean (\pm s.e.) of genetic distance, D , for six regions over 36 populations of *H. spontaneum* (Krasnovodsk plateau is excluded as it is represented by only one population)

Regions	Kopet-Dag mountain range	Kopet-Dag foothill plain	Karakum Desert	Kugitang mountain range	Kugitang foothill plain	Badhyz plateau	Mean
Kopet-Dag mountain range	0.096 \pm 0.003	0.090 \pm 0.007	0.089 \pm 0.008	0.089 \pm 0.009	0.083 \pm 0.008	0.094 \pm 0.008	0.090
Kopet-Dag foothill plain		0.058 \pm 0.007	0.058 \pm 0.005	0.068 \pm 0.005	0.067 \pm 0.005	0.072 \pm 0.007	0.069
Karakum Desert			0.063 \pm 0.012	0.054 \pm 0.009	0.051 \pm 0.008	0.077 \pm 0.008	0.065
Kugitang mountain range				0.015 \pm 0.002	0.029 \pm 0.002	0.053 \pm 0.012	0.051
Kugitang foothill plain					0.029 \pm 0.003	0.044 \pm 0.008	0.051
Badhis plateau						0.110 [†]	0.075

[†]Only two populations compared.

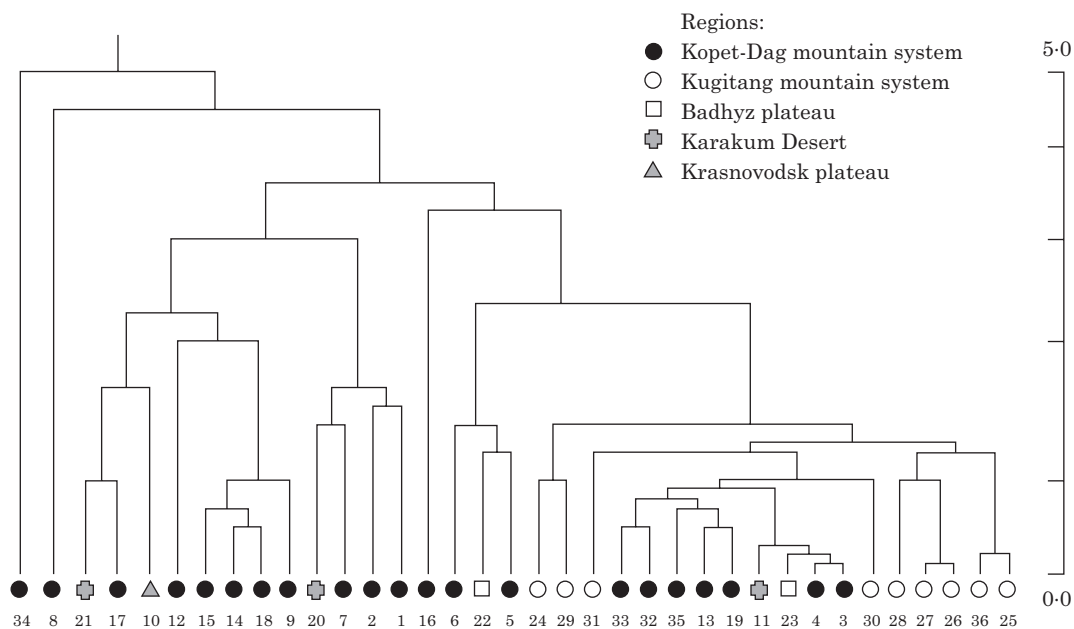


FIG. 2. Dendrogram derived by cluster analysis (method-UPGMA) of Nei's genetic distance values based on allele frequencies in 36 populations of *H. spontaneum*. Mountain system comprises both mountains and foothills.

Genetic distance

Genetic distance was calculated between all pairs of the 36 populations (data not shown). Table 6 presents the mean genetic distance of populations within and between regions. The mean genetic distance between populations was 0.079 (range 0.053–0.192) and between regions was 0.067 (range 0.051–0.090) (Tables 4 and 6). The Kugitang mountain and foothill populations were genetically closer to each other and more distant from all other populations ($P < 0.05$, ANOVA). Populations within each of the other four regions were not genetically more similar to each other than to populations from other regions.

A poor correlation between genetic and geographic inter-population distances was found ($r = 0.14$, $P = 0.035$, Mantel Test). A dendrogram derived by cluster analysis (UPGMA method) of genetic distance between populations also showed that inter-population genetic distance was only partially a function of geographic distance (Fig. 2). The results indicate: (a) populations from the Kopet Dag

mountain system are very different genetically from one another; they do not clump into a single unit but are either clustered into groups consisting of between two and five genetically close populations (irrespective of their mountain or foothill origin) or are distant from all the rest; (b) most populations from the Kugitang mountain system clump into a single compact unit; and (c) populations from both the Badhyz plateau and from the Karakum desert are genetically distinct from other populations from the same region.

Structure of variation and environmental correlates

Total genetic diversity was partitioned into the following components: within population; between populations within a region; and among the six regions (Table 7). The largest single component was within populations (61%), followed by between populations within a region (27%) and among regions (12%). While there was some variation among loci, for the most part the results from each locus are

TABLE 7. Genetic population structure of wild barley in Turkmenistan

Locus	Components of gene diversity			
	H _T	H _C	G _{CS}	G _{ST}
Est-1	0.144	0.102	0.056	0.253
Est-3	0.652	0.355	0.347	0.165
Est-4	0.243	0.163	0.276	0.069
Cat	0.375	0.185	0.406	0.170
GP	0.062	0.058	0.061	0.014
Gdh	0.017	0.016	0.049	0.012
Pgm	0.075	0.046	0.359	0.042
Pgi-1	0.101	0.070	0.273	0.049
Pgi-2	0.033	0.031	0.047	0.008
6 Pgd-1	0.168	0.147	0.100	0.029
6 Pgd-2	0.096	0.086	0.053	0.046
Mdh-1	0.116	0.084	0.249	0.035
Mdh-2	0.075	0.042	0.427	0.028
Mean	0.166	0.107	0.274	0.116
s.e.	0.030	0.008		

Total variability (H_T) is partitioned into three levels (H_C, within population component; G_{CS}, proportion of inter-population differentiation within a region, and G_{ST}, proportion of inter-region differentiation).

consistent with the within population component being the largest and among regions the smallest.

Significant correlation was found between several environmental parameters and genetic variation (all $P < 0.05$). Specifically, percent humidity was negatively correlated to n_a , P and H_e (d.f. = 34, $r = -0.34$, -0.38 and -0.42 , respectively), annual temperature with n_a ($r = 0.44$) and H_e ($r = 0.51$) and longitude with P ($r = 0.33$), H_e ($r = 0.33$) and D ($r = -0.35$). Climatic and geographic factors significantly explained a substantial amount of variation in allele frequencies in four of the 13 loci and total H_e when all loci were analysed by multiple regression analysis (Table 8). Of these, only one locus, Est-1 at the 0.001 level of significance, had a meaningful R^2 (0.76 and 0.64 for the two alleles); the other loci, while significant, had relatively low R^2 s and their biological importance is questionable.

DISCUSSION

Our results indicate a difference in the extent and structure of genetic variation between populations of *H. spontaneum* from the Middle East and Central Asia. The level of genetic diversity (H_e) found in Turkmenian populations of *H. spontaneum* (0.106) is similar to that found by Nevo *et al.* (1986a) in two countries in the Near East, Israel (0.103) and Turkey (0.099), but significantly lower than the level these authors reported for Iran (0.130) and the genetic diversity reported by Jana and Pietrzak (1988) for Turkey (0.14), Jordan (0.17) and Syria (0.15) ($P < 0.05$, ANOVA). While there was no one-to-one match of loci from this study and the previous studies, all loci in this study were also used by Nevo *et al.* (1986a) and most were used by Jana and Pietrzak (1988). Expected heterozygosity, H_e, averaged over all populations, however, is not an adequate measure

TABLE 8. Coefficients of multiple regression (R^2) with H_e and allele frequencies at four loci in 36 Turkmenian populations of *H. spontaneum* as the dependent variables and climatic and geographic factors as the independent variables*

allele	X ₁ X ₂ X ₃	R ² ₁	R ² ₁₂	R ² ₁₂₃
He	Tj	0.26**		
Est-1				
b	EvRn	0.62***	0.76***	
c	EvRn	0.47***	0.63***	
Est-4				
a	TjEv	0.20***	0.32*	
b	TjEv	0.35***	0.44*	
c	TmAIRn	0.22**	0.35*	0.47*
Mdh-1				
b	Tj	0.24**		
Mdh-2				
b	AIRn	0.30***	0.36*	

*Climatic values were taken from the USSR climate reference book. Part 30. Turkmenistan vols 2,4,5 (1967, 1968, 1969) and from multiple-year records of the Meteorological Service of Turkmenistan. AL, altitude; Tm, mean annual temperature; Tj, mean January temperature; Rn, mean annual rainfall; Ev, annual evaporation.

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

of a meta-regional (i.e. Middle East and Central Asia) genetic diversity, comprising within and between population components, especially if meta-regional genetic structures differ. Comparison of total genetic diversity of meta-regional gene pools (H_T) is more appropriate because H_T represents variation in all hierarchical levels. We found H_T of wild barley in Central Asia to be lower than in the Near East, including Israel, Turkey and Iran (Nevo, 1986a) (0.17 vs. 0.21). We are confident in stating that allozyme diversity of wild barley from Central Asia is lower than in the Middle East and eastern Mediterranean, as is RAPD diversity (Volis *et al.*, unpubl. res.).

The pattern of genetic variation found in populations from Central Asia was different to that found in populations from the Middle East and eastern Mediterranean. Nevo *et al.* (1986a, c) and Jana and Pietrzak (1988) found a mosaic or patchy pattern of allele distribution with most alleles being either unique or locally common to a population and/or region, or widespread and common, but not ubiquitous, among the various regions. In this study, which covers a geographic area almost equal to that of previous studies from the Middle East, we also found a mosaic pattern of allozyme variation with noticeable differences between populations over short distances. However, there were the following differences between the studies: (a) of the 13 loci examined, 11 had ubiquitous alleles which were usually the highest frequency alleles in all populations; Nevo *et al.* (1986a) found only two ubiquitous alleles in the 27 loci that they examined; (b) populations from Central Asia contained many widespread alleles, but usually in lower frequencies than reported for the Middle East (Nevo, 1986a); (c) only one of the 43 alleles was unique to a single population as opposed to 12 out of 55 alleles for populations from Turkey (Nevo *et al.*, 1986b; $P < 0.01$, G-test) and 13 out of 76 alleles from populations from

Israel (Brown *et al.*, 1978; $P < 0.05$, G-test); (d) the partitioning of genetic variation into within populations, between populations of a region and among regions differed from that reported by Nevo *et al.* (1986a) (see below).

The different pattern of genetic variation in barley from Central Asia is reflected in the population genetic distances. The average genetic distance between populations of *H. spontaneum* in Central Asia was 0.079. This relatively low genetic distance is consistent with a population structure characterized by a large number of ubiquitous alleles in high frequencies, many secondary widespread or common alleles in relatively low frequency, and a small number of unique or localized rare alleles. The average genetic distances, calculated by Nevo *et al.* (1986a, b) for three countries in the Near East, Israel, Turkey and Iran, were 0.119, 0.110 and 0.065, respectively. Greater within-regional population distances in these countries as compared with Central Asia (except Iran) are coupled with high between-regional population distances (0.134, 0.129 and 0.111 for Israel-Turkey, Israel-Iran and Turkey-Iran, respectively), consistent with the existence of few ubiquitous alleles but many unique or locally common alleles.

In this study, 61% of the total genetic variation was found within populations, 27% between populations of a region and 12% among the six regions. Nevo *et al.* (1986a) reported 54% of the genetic variation was due to within population differences, similar to this study, with 39% of the variation originating between populations of a region and only 8% among the three large Mediterranean regions (Israel, Turkey and Iran). On the other hand, Brown *et al.* (1978) reported 51, 32 and 17% for the same three components of allozyme variation, but the regions were smaller areas of different climates in Israel. The scale of population sampling in our study was intermediate between these two, as was the size of the inter-regional component of variation. The within-population component of variation found for *H. spontaneum* in Turkmenistan seems to represent the most important difference between the Mediterranean and Central Asian ranges of its distribution.

We also found a different pattern from Nevo *et al.* (1986a, b) in the relationship between allele frequencies and environmental parameters. They found a significant relationship between many alleles and environmental parameters and concluded that local selection plays an important role in maintaining the genetic structure in these populations. We found fewer significant relationships and, as most had relatively small R^2 s, their importance as determinants of the extent and structure of genetic variation in populations, even assuming the adaptive nature of allozyme markers, is questionable.

Overall, this study indicates that the mosaic pattern of genetic variation in populations of *H. spontaneum* is a common phenomenon throughout its range. However, we found it less pronounced in the Central Asian part of the species' distributional range. In this area one ubiquitous allele in most loci with secondary widespread alleles in relatively low frequencies was often the norm. There are two possible explanations. Lower genetic variation may be a consequence of the relatively recent colonization of Central Asia by wild barley, with concomitant founder

events, demographic stochasticity and bottlenecks. Alternatively, it may be related to environmental differences between the two regions. The relatively mild climates of the Middle East and eastern Mediterranean as opposed to the harsher, more extreme continental or mountain climate of Central Asia, may allow more genetic differentiation to occur in geographically close populations via local adaptation. In the Middle East, populations separated by only 100 km may experience totally different environmental conditions, from true Mediterranean to desert, and may be governed by different selective pressures. In contrast, populations from Central Asia, where the environment is much harsher, undergo strong macro-environmental stresses that may prevent selection on a smaller scale and genetic drift from being as effective.

Due to the higher within-population component of variation in wild barley from Central Asia, different collection strategies may be needed as compared to the Middle East. When sampling in the Middle East, emphasis should be on collecting many populations within a few broad regions, while sampling in Central Asia requires fewer populations per region.

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