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Worldwide Patterns of Genetic Variation Among Four Esterase Loci in Barley (*Hordeum vulgare* L.)*

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Summary. Electrophoretic assays of 1506 accessions of domestic (*Hordeum vulgare* L.) and wild (*H. spontaneum* Koch.) barley, maintained in the USDA World Barley Collection, led to the following conclusions: (1) worldwide the four esterase loci, *Est 1*, *Est 2*, *Est 3*, and *Est 4*, have a minimum of 7, 12, 6, and 7 alleles, respectively; (2) little or no genetic differentation has developed between *H. vulgare* and *H. spontaneum* at these four esterase loci; (3) substantial genetic polymorphism and heterozygosity occur within many of the accessions despite the heavy inbreeding which results from the mating system of predominant self fertilization and from genetic drift associated with maintenance in small populations; (4) patterns of geographical distribution of alleles at these four loci are not at random over both small and large geographical areas, including differences on a continental scale; (5) four among 16 four-locus combinations of alleles are found in excess and all other combinations occur in deficiency on a worldwide basis.

Key words: Barley – Esterase loci – Genetic polymorphism – Geographical distribution

Introduction

Electrophoretic studies using esterase loci have been useful in monitoring genetic changes that have occurred in experimental barley populations and in obtaining quantitative measures of the factors responsible for the observed changes (e.g., Allard et al. 1972; Kahler 1973; Clegg et al. 1978). Analyses of the reproductive cycle in these experimental barley populations have shown that the outcrossing rate averages less than 1% (Allard et al. 1972) and that the mating system itself is subject to evolutionary change (Kahler et al. 1975). In addition, multilocus analyses of the experimental populations have shown that striking nonrandom associations of alleles develop over loci, i.e. the populations develop a highly organized genetic structure featuring multilocus gene complexes (Weir et al. 1972, 1974; Clegg et al. 1972; Kahler 1973). Similar multilocus complexes have been found in studies of natural populations of the wild relative (*H. spontaneum* Koch.) of domestic barley (Brown et al. 1977).

This paper presents results of a survey of electrophoretically detectable variation in the world collection of domestic barley (*Hordeum vulgare* L.) and wild barley (*H. spontaneum* Koch.) maintained by the United States Department of Agriculture (USDA). The specific objectives were to determine: (1) allelic (allozyme) frequencies for the four esterase loci (*Est 1*, *Est 2*, *Est 3*, *Est 4*) within accessions of the barley collection; (2) how genetic variation is distributed among different populations and/or geographical areas; (3) whether alleles among these four esterase loci are associated in multilocus complexes, and if so, how the multilocus types are distributed on a worldwide basis.

Materials and Methods

The world collection of diploid (2n = 14) barley (*H. Vulgare* and *H. sponteneum*), maintained by the USDA in Beltsville, Maryland, was the seed source for this study. The entries in this collection are maintained as small populations in Beltsville. An entry received as a single unthreshed spike is maintained as a small population derived from the original single spike. Entries received as threshed seed are sown and components (if any) are selected and maintained as separate subpopulations. As an example, when a collection received as threshed grain is sown and found to contain types differing in row-type, growth habit, kernel color, and so forth, each type is harvested, separated and thereafter maintained as more-or-less uniform lines for these particular gross morphological characters. Each subpopulation may, however, be polymorphic

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and/or heterozygous for numerous other characters. Certain materials, such as seed from bulk populations, are maintained as mixed populations to preserve the maximum amount of variability within single entries.

The first time an introduction or its components are grown, a seed sample is taken and reserved for subsequent increases (head samples are also taken for reference). The usual procedure for increasing the seeds is to grow one 10-foot (≃ 3 m) row. Usually, each entry is regrown once every 5 years to rejuvenate and to replenish the seed stocks. Accessions of *H. spontaneum* that cannot be sown or harvested in the usual way are increased in the greenhouse or in hill plantings to facilitate hand harvesting. Thus, it can be assumed that the degree of heterozygosity in each population depends on the kind of sample received. Also, the entries in the collection are expected to be less variable genetically, due to selection and genetic drift, than the populations from which they were derived. In 1968, the entire winter-cultivated (*H. vulgare*) barley collection of 1,295 entries, the spring-cultivated barley collection of 6,587 entries, and 256 samples of wild barley (*H. spontaneum*) from Israel and Turkey, were made available by Dr. J. C. Craddock, Crops Research Division, Agricultural Research Service, (USDA). In 1971, Dr. Craddock (personal communication) also supplied much of the above information concerning the methods by which the collection is maintained.

The methods of horizontal starch gel electrophoresis, including details of sample preparations and staining methods used to reveal allozymes of the four esterase loci (*Est 1*, *Est 2*, *Est 3*, *Est 4*) considered in this study, have been described in detail by Kahler and Allard (1970). Locus designations follow the nomenclature suggested by Kahler et al. (1980). A standard barley variety of known banding pattern ('Atlas') was included in each gel to aid in establishing migrational distances (in cm from the origin) of the allozymes. When small differences in migration were observed between bands, extracts of the two accessions were mixed and electrophoresed to determine mobility differences. This method proved to be reliable in that conclusions regarding allelism reached from mixing experiments were, when checked by progeny tests, invariably supported. Experiments are now underway to verify by segregation tests any allozymes which have thus far been inferred only from mixing experiments.

Table 1. Alleles and their migration ranges (in cm from the origin) for the *Est 1*, *Est 2*, *Est 3*, *Est 4* loci in barley

Locus	Alleles	Migration range
Est 1	0.2	(0.1–0.5)
	1.0	(0.6–1.4)
	1.8	(1.5–1.9)
	2.0	(2.0–2.1)
	2.3	(2.2–2.4)
	2.6	(2.5–2.8)
	0.0	(Null)
Est 2	1.4	(0.8–1.4)
	1.6	(1.6)
	1.8	(1.7–1.8)
	2.0	(1.9–2.1)
	2.3	(2.2–2.4)
	2.7	(2.5–2.8)
	3.0	(2.9–3.2)
	3.4	(3.3–3.5)
	3.6	(3.6–3.7)
	3.9	(3.8–3.9)
	4.0	(4.0–4.3)
	0.0	(Null)
Est 3	3.9	(3.8–4.0)
	4.4	(4.1–4.5)
	4.9	(4.6–5.0)
	5.4	(5.1–5.5)
	5.8	(5.6–5.9)
	0.0	(Null)
Est 4	5.5	(5.2–5.6)
	6.2	(5.8–6.2)
	6.4	(6.3–6.4)
	6.5	(6.5)
	6.6	(6.6)
	6.8	(6.7–6.8)
	0.0	(Null)

Allelic Variability

Thirty-two alleles were found at the *Est 1*, *Est 2*, *Est 3*, *Est 4* loci in our survey of the USDA World Barley Collection. Twenty-eight of the alleles were expressed in codominant fashion (banded) and four were found to be recessive nulls (no band). Although many additional esterase isozymes were observed, they are not considered further because their inheritance is unknown.

Despite careful standardization, there was some variability from gel to gel in migration distances. Consequently, bands falling within specified ranges of migration distances were considered to be the same, e.g., at the *Est 1* locus all bands falling in the range 0.1 to 0.6 cm from the origin are designated as being due to allele 0.2 and all bands falling in the range 2.5 to 2.8 cm are designated as being due to allele 2.6. These migration ranges were established from mixing experiments and experience in reading gels. Table 1 gives a list of alleles and migration ranges for the four esterase loci. In some instances grouping in this fashion may result in more than one allele being placed in a single class and consequential underestimation of the actual number of alleles. However, a study designed to detect hidden allozyme variation in barley (Shumaker et al., unpublished) has shown that cryptic alleles are rare. The present study has therefore probably detected nearly all of the esterase variability in barley.

Figure 1 gives a schematic representation of the esterase allozymes and potential allozymes that were observed. Seven alleles were found at the *Est 1* locus. Five alleles (0.2, 1.0, 1.8, 2.0, 2.6) were verified by progeny tests to be codominant, one allele was identified in progeny tests as a recessive null (0.0, no band), and the remaining potential allozyme, at the 2.3 cm position, was determined to differ from known allozymes by examining composite sample extracts.

The *Est 2* locus has 11 single-banded allozymes and a

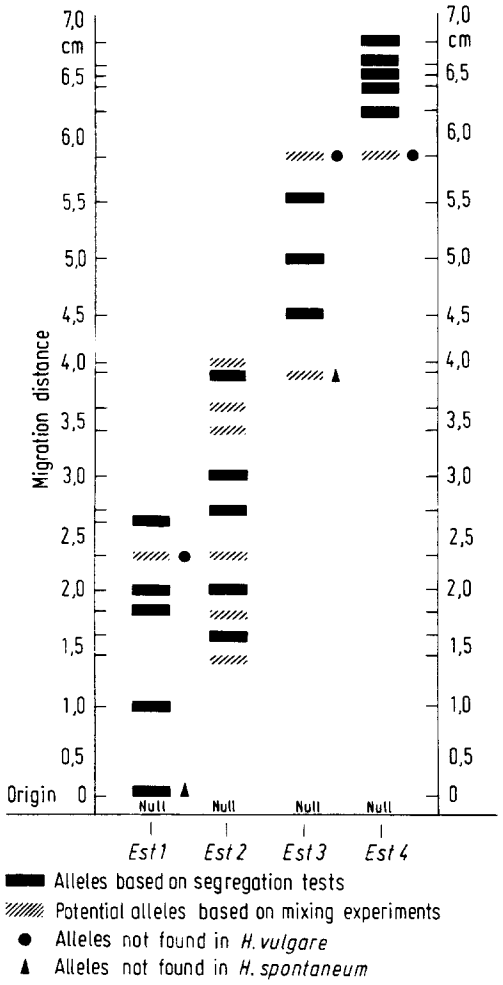

Fig. 1. Schematic representation of esterase allozymes observed in the world collection of domestic (*H. vulgare*) and wild (*H. spontaneum*) barley

recessive null allele (0.0). Five alleles (1.6, 2.0, 2.7, 3.0, 3.9) were verified by progeny tests to be codominant and remaining alleles (1.4, 1.8, 2.3, 3.4, 3.6, 4.0) were identified by mixing plant extracts.

The *Est 3* locus has five double-banded allozymes (only the faster migrating band is shown in Figure 1) and a recessive null allele (0.0). Three alleles, designated by the faster migrating band (4.4, 4.9, 5.4), were verified by progeny tests to be codominant, and two alleles (3.9, 5.8) were identified by mixing of plant extracts.

The *Est 4* locus has six double-banded expressions (only the faster migrating band is shown in Figure 1) and a recessive null allele (0.0). Five alleles, designated by the faster band (6.2, 6.4, 6.5, 6.6, 6.8), were verified by progeny tests to be codominant. A unique six-banded type (lead bands are at 5.5, 6.2, and 6.6 cm) was observed in one collection (P.I. 296897) from Eshtaol, Israel. This is possibly a fixed 'triplicate' genotype.

In total 22 alleles have been verified at these four esterase loci by progeny tests and 10 alleles have been identified by mixing experiments. Two alleles (*Est 1-0.0*, *Est 3-3.9*), observed in the domestic barley collection, were not found in the wild barley collection and three alleles (*Est 1-2.3*, *Est 3-5.8*, *Est 4-5.5*) found in the wild barley collection were not observed in the domestic barleys. Otherwise, all other esterase alleles were observed in both *H. vulgare* and *H. spontaneum* collections.

Allelic Distribution Patterns

Single-locus allelic frequencies were determined by assaying four or more individuals in each of 1,506 accessions. The accessions assayed were selected to cover the widest available range of geographic variability in both the domestic collection (1,358 accessions from 37 countries) and the wild barley collection (148 accessions from Israel and Turkey). The sample of wild barley may not be representative because the limited materials available do not cover the range of distribution of *H. spontaneum*, which extends from the eastern Mediterranean to Afghanistan. All 32 alleles described above and shown in Figure 1, including the alleles identified by mixing experiments, were included in the analyses.

Table 2 lists the 37 countries, the number of accessions and the total number of plants that were assayed per country. When fewer than 50 accessions were available per country, all accessions were assayed. When more than 50 accessions were available for a country, a random sample of 50 (in a few cases 51 to 57) accessions was assayed, except that all of the 448 accessions from the USA were assayed. The distribution of genetic variability could not be determined within countries because information was often not available concerning the precise locations where collections were made. Data are given on a country-by-country basis only for the *Est 1* locus (Table 2), and for the *Est 2*, *Est 3* and *Est 4* loci data are given on a continental or subcontinental basis (Tables 3-5).

Table 2 shows that three of the seven alleles (0.2, 1.0, 1.8) observed at the *Est 1* locus occur in substantial frequencies worldwide, whereas four of the alleles are rare. Worldwide the frequencies of the three common alleles were approximately 0.27, 0.19, and 0.50, respectively. Thus, they account for 96% of the total variability at this locus in domestic barley. Allele 2.6 is locally common (0.18) in the East Asian area (partly replacing alleles 0.2 and 1.0) and in the Middle South Asian (0.11) area (at the expense of allele 1.8) but allele 2.6 is nearly absent in all other areas.

The allelic frequency distribution of the 12 alleles at the *Est 2* locus is given in Table 3. This locus is unusual in two respects: (1) the number of alleles is exceptionally

Table 2. Allelic frequencies at the *Est 1* locus in the World Barley Collection (*Hordeum vulgare* and *H. spontaneum*). N^a (C.I.) gives the total number of cereal inventory (C.I.) accessions sampled in each location. N^b (plants) refers to the total number of plants assayed

Location	N^a (C.I.)	N^b (plants)	Alleles						
			0.2	1.0	1.8	2.0	2.3	2.6	0.0
Finland	7	31			1.00				
Sweden	52	248	0.48	0.07	0.45				
Denmark	12	64	0.08		0.84			0.08	
England	44	184	0.50	0.11	0.39				
Poland	50	221	0.50		0.50				
Ireland	5	19		0.20	0.80				
France	50	220	0.39	0.08	0.53				
N. & W. Europe	220	987	0.42	0.06	0.51			0.01	
Spain	30	144	0.06	0.44	0.50				
Italy	9	50	0.33		0.67				
Rumania	3	18			1.00				
S. Europe	42	212	0.11	0.32	0.57				
USSR	49	222	0.38	0.18	0.40	0.02		0.02	
Cyprus	10	43	0.10	0.20	0.70				
Turkey	49	225	0.31	0.22	0.47				
Iraq	25	125	0.56	0.12	0.12	0.20			
Israel	7	58	0.12	0.25	0.63				
Greece	3	21	0.25		0.75				
S. W. Asia	94	472	0.33	0.19	0.43	0.05			
Central Asia	9	28	0.50	0.10	0.30			0.10	
Iran	51	240	0.19	0.32	0.38			0.09	0.02
Afghanistan	46	193	0.28	0.26	0.21			0.25	
India	49	207	0.15	0.56	0.29				
Middle S. Asia	155	668	0.23	0.36	0.29			0.11	0.01
Manchuria	3	16			0.67			0.33	
Korea	46	186	0.11		0.74			0.15	
Japan	57	236	0.12	0.08	0.46			0.34	
China	53	220	0.14	0.17	0.66			0.03	
E Asia	159	658	0.12	0.09	0.61			0.18	
Egypt	51	227	0.20	0.20	0.60				
Ethiopia	46	177	0.41	0.47	0.12				
N. E. Africa	97	404	0.30	0.32	0.38				
S. Africa	14	65	0.13	0.47	0.40				
Australia	39	153	0.28	0.16	0.56				
USA	448	1672	0.21	0.18	0.58	0.01		0.02	
Canada	9	52	0.56		0.33			0.11	
N. America	457	1724	0.21	0.17	0.58	0.01		0.03	
Mexico	9	50	0.11	0.33	0.56				
Guatemala	5	14	0.20	0.80					
C. America	14	64	0.15	0.50	0.36				
Venezuela	6	37	0.43	0.57					
Chile	2	11	0.50		0.50				
Argentina	10	57	0.40	0.40	0.20				
S. America	18	105	0.42	0.42	0.16				
Total (*H. vulgare*)	1358	5734	0.27	0.19	0.50	0.01		0.03	
Israel & Turkey (*H. spontaneum*)	148	608	0.03	0.09	0.84	0.01	0.02	0.01	

Table 3. Allele frecuencies at the *Est 2* locus

Location	N[a] (C.I.)	N[b] (Plants)	Alleles											
			1.4	1.6	1.8	2.0	2.3	2.7	3.0	3.4	3.6	3.9	4.0	0.0
N. & W. Europe	220	987	0.03	0.02	0.03	0.04		0.80	0.00			0.07		0.01
S. Europe	42	212	0.07	0.02				0.89				0.02		
U.S.S.R.	49	222			0.08			0.76		0.02		0.10		0.04
S. W. Asia	94	472		0.01	0.03	0.04		0.87	0.02		0.01	0.02		
Middle S. Asia	155	668			0.01	0.04		0.64	0.02		0.01	0.10		0.18
E. Asia	159	658				0.02		0.37				0.29		0.32
N. E. Africa	97	404			0.01	0.01		0.92	0.03		0.01	0.01		0.01
S. Africa	14	65						0.93					0.07	
Australia	39	153	0.03			0.07		0.82				0.05		0.03
N. America	457	1724		0.00	0.05	0.01	0.01	0.75	0.01	0.00	0.00	0.12	0.00	0.05
C. America	14	64	0.07					0.86				0.07		
S. America	18	105	0.06					0.83				0.11		
Total (*H. vulgare*)	1358	5734	0.01	0.01	0.02	0.02	0.01	0.73	0.01	0.00	0.00	0.11	0.00	0.08
Israel & Turkey (*H. spontaneum*)	148	608	0.05	0.02	0.03	0.12	0.01	0.65	0.04	0.01	0.01	0.01	0.01	0.04

[a], [b] See Table 2

Table 4. Allele frequencies at the *Est 3* locus

Location	N[a] (C.I.)	N[b] (plants)	Alleles					
			3.9	4.4	4.9	5.4	5.8	0.0
N. & W. Europe	220	987		0.17	0.58	0.25		
S. Europe	42	212		0.11	0.47	0.42		
U.S.S.R.	49	222		0.14	0.59	0.25		0.02
S. W. Asia	94	472		0.08	0.48	0.43		0.01
Middle S. Asia	155	668		0.11	0.39	0.44		0.06
E. Asia	159	658		0.26	0.59	0.14		0.01
N. E. Africa	97	404		0.06	0.46	0.46		0.02
S. Africa	14	65			0.54	0.46		
Australia	39	153		0.17	0.56	0.27		
N. America	457	1724	0.00	0.18	0.54	0.27		0.01
C. America	14	64		0.07	0.36	0.57		
S. America	18	105		0.21	0.42	0.37		
Total (*H. vulgare*)	1358	5734	0.00	0.16	0.52	0.30		0.02
Israel & Turkey (*H. spontaneum*)	148	608		0.43	0.21	0.25	0.04	0.07

[a], [b] See Table 2

Table 5. Allele frequencies at the *Est 4* locus

Location	N[a] (C.I.)	N[b] (plants)	Alleles[c]					
			6.2	6.4	6.5	6.6	6.8	0.0
N. & W. Europe	220	987	0.04	0.68	0.11	0.11		0.06
S. Europe	42	212		0.66		0.21		0.13
U.S.S.R.	49	222	0.04	0.62	0.02	0.22	0.04	0.06
S. W. Asia	94	472	0.06	0.42	0.07	0.28		0.17
Middle S. Asia	155	668	0.02	0.56	0.02	0.19		0.20
E. Asia	159	658	0.02	0.64	0.08	0.11		0.15
N. E. Africa	97	404	0.02	0.73	0.06	0.12		0.07
S. Africa	14	65	0.07	0.20	0.07	0.26		0.40
Australia	39	153	0.14	0.65	0.02	0.17		0.02
N. America	457	1724	0.01	0.68	0.01	0.21	0.01	0.08
C. America	14	64		0.60	0.07			0.33
S. America	18	105	0.09	0.38		0.24		0.29
Total (*H. vulgare*)	1358	5734	0.03	0.63	0.05	0.18	0.00	0.11
Israel & Turkey (*H. spontaneum*)	148	608	0.02	0.65	0.02	0.25	0.01	0.05

[a, b] See Table 2

[c] A seventh allele (5.5) was observed in a fixed 'triplicate' genotype in an *H. spontaneum* collection (P.I. 296897) from Eshtaol, Israel

large; and (2) one of the 12 alleles, allele 2.7, is sufficiently frequent in most areas that it might be characterized as a 'wild type' allele. Again, as with the *Est 1* locus, the East Asian and Middle South Asian areas are unusual. In these two areas allele 2.7 drops much below its worldwide frequency and the frequencies of alleles 0.0 and/or 3.9 rise much above their worldwide frequencies.

Six alleles were observed at the *Est 3* locus (Table 4). This locus is characterized by the high frequency of the 4.9 allele and the relatively high frequency of the 5.4 allele, which were present in approximately 50 and 25% frequency, respectively, in nearly all of the major barley-growing areas of the world. Once again the East Asian and Middle South Asian areas were unusual. Allele 4.9 was low in frequency in Middle South Asia, the frequency of allele 5.4 was low in East Asia, and there was some indication that allele 0.0 was more common than usual in Middle South Asia.

The frequency distributions of the six alleles observed at the *Est 4* locus are given in Table 5. This locus is characterized by the similarity of the frequency distributions over the major barley-growing areas. Thus (aside from areas where small sample sizes may have led to random departures) allele 6.4 is consistently frequent in all areas, allele 6.6 is consistently moderately frequent, alleles 6.2, 6.5, and 0.0 occur consistently in low frequency, and allele 6.8 is rare or absent in all areas.

Genetic Identity and Cluster Analysis

Genetic identity values (Nei 1972) were calculated for each country and subjected to cluster analysis (Sokal and Sneath 1963). Observed genetic identity values (Kahler 1973) ranged from 0.525 to 0.989, supporting the conclusion reached from the frequency distributions given in Tables 2-5 that allelic frequencies are heterogeneous geographically. The results of five rounds of cluster analysis of the data for the 24 countries for which 10 or more accessions had been assayed are presented in Table 6. The results indicate that cultivated barley consists of two subgroups, a Western subgroup and an Asiatic subgroup. Denmark was the only country which remained independent of Clusters A and B by the fifth round of clustering. This may be a sampling effect associated with the small number of Danish accessions assayed.

Table 6. Country clusters in order of similarities of average genetic identities after five rounds of cluster analyses

Cluster A:	Sweden, France, Central Asia, England, U.S.S.R., Cyprus, Egypt, Turkey, Australia, U.S.A., Spain, India, Ethiopia, Iran, Israel, Poland, Italy, South Africa, Iraq
Cluster B:	Japan, China, Korea, Afghanistan
Cluster C:	Denmark

Genetic Polymorphisms

Many of the accessions of this study were polymorphic, i.e., more than one genotype was found within the accession. To obtain a quantitative measure of the extent of polymorphism, accessions which were genetically invariant for the four esterase loci (i.e., included only a single genotype) were classified as monomorphic and accessions which included two or more homozygotes and/or heterozygotes were classified as polymorphic. The distribution of polymorphisms over locations was measured as

$$\chi^2 = \Sigma \frac{(O_{1-4} - \bar{P} \cdot N)^2}{\bar{P} \cdot N},$$

where O is the observed number of polymorphic accessions in a given location, N is the number of accessions sampled for the location, $\bar{P} = \Sigma \frac{(O_{1-4})}{N}$ and $\bar{P} \cdot N$ is the expected number of polymorphic accessions per location.

Table 7 gives the number and distribution of observed and expected polymorphic accessions for each locus, and over all loci, for each of 12 major barley-growing regions, and for *H. spontaneum*. Proportions of polymorphic accessions for each locus, and over all loci, can be determined from the numbers given in Table 7. Chi square values for the *Est 1*, *Est 2*, and *Est 3* loci were nonsignificant, an observation which indicates that polymorphic accessions are distributed uniformly for these loci on a worldwide basis. However, χ^2 for the *Est 4* locus was significant at $P < 0.01$, indicating heterogeneous distribution of polymorphisms for this locus on a worldwide basis. The four-locus heterogeneity chi square value of 47.85 was significant at $P < 0.01$. This heterogeneity results primarily from higher levels of polymorphisms in the Asian accessions. Most of the heterogeneity is associated with the *Est 4* locus, but there is a trend in the other loci toward deficiencies on polymorphism in the European accessions (including North American accessions) and excesses in polymorphism in Asian collections.

Table 7. The number and distribution of observed and expected (in parentheses) polymorphic accessions in 12 collection locations of *H. vulgare* and 148 accessions of *H. spontaneum*

Location[a]	N[b] (C.I.)	N[c] (plants)	Locus				O_{1-4}	$\bar{P} \cdot N$
			Est 1	*Est 2*	*Est 3*	*Est 4*		
N. & W. Europe	220	987	8 (11)	10 (9)	11 (18)	8 (23)	37	(55)
S. Europe	42	212	1 (2)	1 (2)	3 (2)	4 (4)	9	(10)
U.S.S.R.	49	222	2 (2)	2 (2)	4 (3)	5 (5)	13	(12)
S. W. Asia	94	472	2 (5)	2 (4)	2 (5)	16 (9)	22	(23)
Middle S. Asia	155	668	13 (7)	12 (6)	14 (9)	28 (16)	67	(38)
E. Asia	159	658	8 (8)	9 (6)	12 (9)	30 (16)	59	(40)
N. E. Africa	97	404	8 (5)	3 (4)	9 (5)	8 (10)	28	(24)
S. Africa	14	65	1 (1)	0 (1)	0 (1)	2 (1)	3	(3)
Australia	39	153	4 (2)	0 (2)	2 (2)	4 (4)	10	(10)
N. America	457	1724	19 (22)	15 (18)	18 (25)	29 (46)	81	(112)
C. America	14	64	0 (1)	0 (1)	0 (1)	0 (1)	1	(3)
S. America	18	105	0 (1)	0 (1)	1 (1)	3 (2)	4	(4)
Total (*H. vulgare*)	1358	5734	66	54	76	138	334	
Total (*H. spontaneum*)	148	608	10	30	30	18	88	

χ^2 Est $1_{[11]} = 14.47$; χ^2 Est $2_{[11]} = 14.86$; χ^2 Est $3_{[11]} = 13.88$; χ^2 Est $4_{[11]} = 44.66^{**}$; $\chi^2_{1-4[11]} = 47.85^{**}$

[a] See Table 2 for a listing of those countries included in each location
[b/c] See Table 2
** χ^2 values significant at P = 0.01

It is noteworthy that levels of polymorphism were higher for the *Est 1*, *Est 2*, *Est 3*, and *Est 4* loci in the *H. spontaneum* accessions (0.067, 0.203, 0.203, 0.121, respectively) than in the *H. vulgare* accessions (0.049, 0.040, 0.056, 0.102, respectively). Similarly, over all loci, the proportion of polymorphic accessions in the *H. spontaneum* accessions (0.594) was more than double the proportion in *H. vulgare* accessions (0.246) on a worldwide basis. Polymorphism was higher (0.401) in the Asian collections (including Middle South Asia and Eastern Asia) than the overall value (0.246) for cultivated barley. However, the Asian collections were still less polymorphic than the wild barleys.

Heterozygosity

Estimates of the proportion of heterozygotes obtained in this study are given in Table 8. These estimates show that domestic barley, as it is maintained by the USDA, averages < 0.5% heterozygosity for the four esterase loci. The *Est 4* locus is consistently lower in heterozygosity than the other three loci. North American and European collections are generally lower in heterozygosity for loci 1, 2 and 3 (< 0.5%) than the Oriental and Middle S. Asian collections, which range from 0.17% to 1.47%.

Heterozygosity estimates in the wild collections from Israel and Turkey were substantially higher for all loci, including the *Est 4* locus than in the domestic barleys. These estimates support the finding that heterozygosity is higher on the average in natural populations of *H. spontaneum* (Brown et al. 1978; Nevo et al. 1979) than in cultivated barley.

Table 8. Percent heterozygosity observed among groups of countries of the world. See Tables 6 and 9 for a listing of countries included in each group

Location	Locus				Mean over all loci	N[a]
	Est 1	*Est 2*	*Est 3*	*Est 4*		
(*H. vulgare*)						
Cluster A	0.29	0.07	0.27	0.02	0.16	4123
Cluster B	0.67	0.54	0.54	0.00	0.44	746
N. American	0.23	0.06	0.23	0.00	0.13	1710
European	0.19	0.09	0.14	0.05	0.12	2119
Middle S. Asia	1.47	0.73	1.22	0.00	0.86	409
Oriental	0.34	0.17	0.51	0.00	0.25	593
(*H. spontaneum*)						
Israel and Turkey	1.56	2.08	2.60	0.087	1.78	576

[a]N is the total number of plants assayed

Multilocus Gametic Frequency Distributions

Earlier studies of experimental barley populations (Clegg et al. 1972; Weir et al. 1972, 1974; Kahler 1973; Clegg et al. 1978) have shown that non-random associations of alleles at different loci develop rapidly in such populations. Brown et al. (1977) have found similar associations in wild barley populations. To determine whether the alleles of the four loci of this study are associated at random, observed four-locus gametic frequencies were calculated and compared to gametic equilibrium values. The number of four-locus genotypes that is possible with the observed number of alleles (7, 12, 6 and 7 for loci 1, 2, 3, and 4, respectively) is very large. Consequently, each locus was reduced to diallelic state to bring the analysis into manageable proportions. The convention used earlier by Weir et al. (1972) was followed in assigning alleles to classes: one allele (usually the most frequent) was designated Allele 1 and all other alleles were combined into a synthetic class designated Allele 2. The new allelic classes for the multilocus analysis are:

Est 1, Allele 1 = 1.8
Est 2, Allele 2 = 0.2 + 1.0 + 2.0 + 2.6

Est 2, Allele 1 = 2.7
Est 2, Allele 2 = 1.4 + 1.6 + 1.8 + 2.0 + 2.3 + 3.0 + 3.4 + + 3.6 + 3.9 + 4.0 + 0.0
Est 3, Allele 1 = 5.4
Est 3, Allele 2 = 3.9 + 4.4 + 4.9 + 5.8 + 0.0
Est 4, Allele 1 = 6.4
Est 4, Allele 2 = 5.5 + 6.2 + 6.5 + 6.6 + 6.8 + 0.0

Observed gametic frequencies were computed from the sums of appropriate genotypic frequencies following the procedures of Clegg et al. (1972). Expected gametic frequencies were calculated as the products of observed single-locus allelic frequencies. Table 9 gives observed frequencies and relative deviations to nearest integer from expected frequencies for 16 four-locus gametic types in four major geographical regions. Among the 16 gametic types, eight were found in substantial frequency (> 0.05) in one or more regions and the remaining eight were infrequent or rare in all regions. The eight frequent gametic types are 1111, 1121, 1221, 1222, 2111, 2112, 2121, and 2122, and their summed frequencies for the North American, European, Middle South Asian, and Oriental regions are 0.816, 0.868, 0.857, and 0.935, respectively. Note that the 1221 and 1222 four-locus gametic types occur in highest frequency in the Orient, and the 2112 and 2111 types occur in highest frequency in Middle South Asia.

Among the 16 gametic types, four were conspicuously and consistently in excess in all of the four regions. These were the balanced opposite sets of 1221 and 2112 and

Table 9. Observed four-locus diallelic gametic frequencies and relative deviations (in parentheses) from expected frequencies in four major regions

	Regions[b]			
Gametes	North American	European	Middle S. Asian	Oriental
1111	0.076 (7)	0.094 (17)	0.048 (−9)	0.019 (−5)
1112	0.035 (0)	0.019 (−41)	0.007 (−19)	0.001 (−5)
1121	0.160 (−134)	0.159 (−43)	0.054 (−11)	0.079 (−69)
1122	0.126 (48)	0.080 (−18)	0.030 (−9)	0.048 (−14)
1211	0.002 (−28)	0.004 (−19)	0.007 (−6)	0.000 (−16)
1212	0.000 (−14)	0.000 (−11)	0.000 (−6)	0.000 (−5)
1221	0.151 (102)	0.085 (99)	0.112 (47)	0.341 (88)
1222	0.053 (26)	0.027 (18)	0.039 (13)	0.114 (27)
2111	0.116 (68)	0.133 (32)	0.199 (26)	0.068 (29)
2112	0.042 (11)	0.081 (78)	0.145 (29)	0.027 (12)
2121	0.186 (62)	0.176 (−22)	0.164 (−6)	0.213 (42)
2122	0.032 (−53)	0.113 (25)	0.096 (−1)	0.074 (10)
2211	0.000 (−25)	0.004 (−17)	0.025 (−8)	0.003 (−9)
2212	0.000 (−8)	0.002 (−11)	0.017 (−5)	0.002 (−2)
2221	0.008 (−43)	0.005 (−50)	0.021 (−29)	0.007 (−61)
2222	0.013 (−2)	0.016 (−7)	0.035 (−4)	0.004 (−22)
N[a]	1674	1947	574	593

[a]N equals the total number of plants assayed in each region
[b]Regions include the following countries: North American = U.S.A., Can.; European = Fin., Swe., Den., Eng., Pol., Ire., Fra., Spa., Ita., Rum., Cyp., Tur., Irq., Gre., Egp., Eth., U.S.S.R.; Middle S. Asian = C. As., Ira., Afg., Ind.; Oriental = Man., Kor., Jap., Chi.

Table 10. Observed four locus diallelic gametic frequencies and relative deviations to the nearest integer (in parentheses) from expected frequencies in Clusters A and B (see Table 6)

	Country Clusters	
Gametes	A	B
1111	0.077 (6)	0.015 (−8)
1112	0.026 (−65)	0.001 (−7)
1121	0.148 (−170)	0.005 (−76)
1122	0.098 (31)	0.039 (−19)
1211	0.003 (−47)	0.000 (−20)
1212	0.000 (−32)	0.000 (−7)
1221	0.108 (223)	0.314 (112)
1222	0.041 (63)	0.096 (27)
2111	0.138 (133)	0.086 (39)
2112	0.078 (5)	0.034 (13)
2121	0.174 (8)	0.214 (51)
2122	0.082 (−28)	0.078 (8)
2211	0.003 (−48)	0.016 (−11)
2212	0.001 (−29)	0.015 (1)
2221	0.009 (−96)	0.006 (−86)
2222	0.013 (−23)	0.031 (−16)
N[a]	4123	746

[a]N equals the total number of plants assayed in each cluster

1222 and 2111, which are the same four-locus sets that have come into excess in experimental populations of barley (Clegg et al. 1972; Kahler 1973; Clegg et al. 1978). The results of a parallel multilocus analysis for the two groups of countries obtained by cluster analysis are given in Table 10. Again the 1221, 2112, 1222 and 2111 gametic types are present in high frequency and they are also present in excess. The 1221 and 1222 types are characteristic of Cluster B (Orient) and the 2112 and 2111 types of Cluster A (Occident).

Discussion and Conclusions

It is clear from this assay of 1,506 accessions of domestic and wild barley that substantial genetic variability for esterase allozymes exists among accessions in the USDA World Collection. The results also indicate that there is little, if any, difference between wild and cultivated barleys respecting esterase allozymes. This supports the notion (Harlan and Zohary 1966; Zohary 1970) that the two taxonomic groups constitute a single species (*H. vulgare L.*) and that the main differences between the two groups are in characters associated with domestication. A study designed to detect hidden allozyme variation in barley (Shumaker et al. unpublished) has revealed, as noted earlier, that cryptic alleles are rare. It therefore seems unlike-

ly that discovery of hidden allozymes will alter the conclusion that wild and cultivated types are very similar regarding electrophoretically detectable variation.

This study also makes clear that substantial genetic variability exists within many accessions in the world collection despite the heavy inbreeding which is known to result from the mating system in barley (Allard et al. 1972; Kahler et al. 1975) and the genetic drift associated with repeated growing of the accessions in small populations. It is improbable that this variability is maintained by migration because the world collection is handled in such a way that contaminants resulting from the occasional hybridizations or migrations that occur between entries in the world collection can be identified and eliminated by roguing. A mutation study involving a five-locus survey of 84,126 individuals and 168,252 possible mutational events per locus indicates that the pooled mutation rate is $< 1.19 \times 10^{-6}$ (Kahler et al., unpublished). Thus, it is also unlikely that the variability in these accessions results from spontaneous mutation. By elimination, we conclude that some type of balancing selection is responsible for the observed within-accession variability.

Analysis of geographical variation indicates that European and North American collections are similar to each other but differ from Asian collections in allelic frequencies at some loci. The *Est 1-2.6* allele, which is common in the East Asian (0.18) and Middle South Asian (0.11) regions and absent or rare in all other regions, is a particularly clear-cut example. Cluster analysis of genetic identity values also separates cultivated barleys into two main groups, a Western and an Asian group. Analysis of four-locus gametic frequencies revealed that four gametic types, the balanced opposite 1221 and 2112 and 1222 and 2111 sets, were consistently in high frequency and that their frequencies were also consistently higher than expected assuming random associations among alleles at different loci. The multilocus analyses also revealed that the 1221 and 1222 gametic types were characteristic of the Asian cluster and the 2112 and 2111 types were characteristic of the Western cluster. Whether the observed distributional patterns are due to selection, differing patterns of migration from the center of origin of barley in the Eastern Mediterranean Region, or founder effects, cannot be determined from the present data.

Comparisons of the levels of genetic polymorphism and heterozygosity over locations showed that Oriental barleys are relatively more polymorphic but not more heterozygous than Occidental barleys, whereas wild barley is both more polymorphic and more heterozygous than domestic barley. This difference between wild and cultivated barley may have its basis in the effects of domestication itself on reducing genetic polymorphism (Suneson 1960; Nat. Acad. Sci. Publ. 1972; Harlan 1977; Marshall 1977). Modern plant breeding practices also tend to reduce genetic polymorphism and it may be that the western accessions are less polymorphic than the Oriental accessions because they had been exposed longer to modern selective practices before inclusion in the World Collection than the Asian barleys.

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