Theor Appl Genet (1988) 75:529–538

Natural selection of allozyme polymorphisms: a microgeographic climatic differentiation in wild emmer wheat (*Triticum dicoccoides*)

E. Nevo *, A. Beiles and T. Krugman

Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31999, Israel

Received August 25, 1987; Accepted September 3, 1987
Communicated by H. F. Linskens

Summary. Allozymic variation in proteins encoded by 48 loci was analyzed electrophoretically in 1984 and 1985 in 137 individual plants of wild emmer wheat, *Triticum dicoccoides,* from a microsite in Yehudiyya, northeast of the Lake of Galilee, Israel. The test involved two climatic microniches in the open Tabor oak forest (1) *sunny* between trees and (2) *shady* under trees' canopies. Significant genetic differentiation at single-, two- and multilocus structures was found between neighboring climatic niches, which were only separated by a few meters. Our results suggest that allozyme polymorphisms in wild emmer wheat are partly adaptive, and differentiate primarily at the multilocus level by climatic factors presumably related to aridity stress.

Key words: Allozyme polymorphisms – Natural selection – Microclimatic differentiation – Wild emmer wheat – *Triticum dicoccoides*

Introduction

The problem of the dynamics of allozyme polymorphisms in nature can be explored in many ways: ecologically, biochemically and physiologically (reviewed in Nevo 1983 a). Ecological studies can be conducted either at the macro- (Nevo et al. 1984) or microgeographical level (Allard et al. 1972; Hamrick and Allard 1972; Hamrick and Holden 1979; Nevo et al. 1981, 1982 a, b, 1983, 1986). Our analysis of genetic diversity of wild emmer wheat, the progenitor of all cultivated wheats, began at the macrogeographical regional scale (Nevo et al. 1982 a). Regional-results indicated that wild emmer is genetically variable, and that genetic differentiation of populations included regional and local patterns, with sharp genetic differentiation over short distances. Climatic and soil factors appeared to be important ecological differentiators of the genetic structure of wild emmer populations. We later investigated detailed microgeographical genetic differentiation (Golenberg and Nevo 1987; Golenberg 1986).

Multidisciplinary studies conducted on wild emmer at the Institute of Evolution until 1983 have been reviewed by Nevo (1983 b). The population genetics of wild emmer, based on 50 electrophoretically tested gene loci, was described by Nevo et al. (1982 a), and multilocus population and genetic dynamics were studied in depth at the Yehudiyya-Qazrin 10 km transect in the Golan Heights by Golenberg (1986). In a complementary paper we studied *edaphic* selection on allozyme polymorphisms in wild emmer (Nevo et al. 1988). Our objective in the present paper is to unravel genetic differentiation at both single and multilocus structures in wild emmer at a microsite subdivided into two microclimatic niches. We report here both qualitative and quantitative uni- and multilocus differentiation of allozymes at the Yehudiyya microsite in the western lower flanks of the Golan Heights. It is presumed that this differentiation derives from natural selection acting on the allozymes under study (Nevo and Beiles 1988), or their linked genes. Neutrality of these allozymes is therefore directly and/or indirectly negated, and climatic selection is the most likely explanatory model for a considerable amount of the allozymes studied here.

Material and methods

Wild emmer wheat, *Triticum dicoccoides,* (genomic constitution AABB) is the allotetraploid wild progenitor of all bread

* To whom correspondence should be addressed

wheats (Zohary 1970; Feldman 1976). It is distributed over the Near East Fertile Crescent, but its center of distribution is found in the catchment area of the Upper Jordan Valley. In this area wild emmer grows as an annual, highly-selfing, component grass in several steppe-like herbaceous formations in the *Quercus ithaburensis* or in the *Q. brantii* open park forest belts (Zohary 1973). It grows primarily on basaltic and terra rossa soil types. It is restricted mainly to primary habitats growing together with wild barley and wild oats. Wild emmer ranges over a wide altitudinal amplitude. Robust, early maturing phenotypes grow in the winter-warm slopes facing the Sea of Galilee (in our Yehudiyya microsite testing area), and range as low as 100 m below sea level. More slender and late-flowering types occur in higher elevations reaching 1,400 m on Mount Hermon (Zohary 1970).

We assayed electrophoretically 137 individual plants of wild emmer for genetic variation at 48 loci tested during two consecutive years 1984 and 1985. Sampling was done in an open oak park forest of *Quercus ithaburensis* at the Yehudiyya site in the lower foothills of the Golan Heights, northeast of the Lake of Galilee. The altitude of the site is 200 m above Mediterranean sea level, and the area consists of brown basaltic soil (Dan and Nissim 1972). The mean annual rainfall at the site is 678 mm and the mean annual temperature is 19 °C (mean ranges 10.5° and 27 °C in January and August, respectively).

Sampling was conducted in pairs in two microclimatic niches: (1) *In shade,* under the canopy of the oak trees (trees 10–20 m in height, with canopy diameters of up to 20 m); and (2) *In sun,* in the circumference around each tree and between trees. While (1) is largely shaded during daytime, (2) is exposed in daytime to continuous sun radiation and drying. In each separate pair we sampled several plants first in the shade under the tree canopy, and secondly in the nearby intertree spaces fully exposed to the sun and only a few meters apart. In 1984 we assayed electrophoretically 30 plants of wild emmer from six repetitive experiments (i.e. six trees and their neighborhoods) and in 1985 we assayed 107 plants in 12 trees. Altogether we analyzed 137 plants in 18 trees.

We used SPSS-x (1986) and SAS (1985) statistical packages for uni- and multivariate analyses. Specific tests are described for each statistical analysis.

Results

Microclimatic measurements

The following comparative temperature and humidity measurements at the two microclimatic niches, *sunny* (between trees) and *shady* (under tree canopy) were conducted on May 8, 1987 and appear in Table 1. Clearly, all temperature comparisons in air (1 m above ground) and soil surface are significantly higher in the sun as compared to those in the shade. Relative humidity was higher in the shade, but not significantly so in the 10 comparisons made. Presumably, differences in relative humidity will show up if extended over more trees, and/or over the entire day cycle.

In any event, the two microniches vary significantly in their plant formation. The shady microhabitat under the oak canopy harbors the plant formation of *Ricotia lunaria* and *Tordylium aegyptiacum,* with very sparse growth of other species. The sunny microhabitat between the oak trees consists of the plant formation of *Psoralea hirsuta – Carthamus glaucus* with *Ami majus, Olchoria pumilum, Eryngium creticum, Gundelia Tournefortii, Lavatera trimestris* with dense stands of wild cereals, *Triticum dicoccoides, Hordeum spontaneum* and *Avena sterilis.* Wild emmer was very sparse in the shade under the oak canopy, abundant in the sun at the circumference of trees, and less abundant in the sunny spaces between trees.

Allele frequencies

The allele frequencies at 21 polymorphic loci, subdivided according to shade, sun, and year (1984, 1985), are presented in Table 2. The raw data are followed in the same table by χ^2, or Fisher's exact tests.

Distribution of polymorphism among loci. Of the 48 loci, 24 proved to be totally monomorphic (*Aat-1*A, *1*B, *2*B; *Acph-2; Adh-1*B, *2*B; *Cat*-A, B; *Est-4*A; *Gdh*-B; *Ipor* A, B; *Mdh-1*B, *2; Nadh-1*A, B, *2; Pgi*-B; *6Pgd-1*A, B; *Pepc; Pgm*-A; *Pept-1*A, *2*); and 3 were nearly polymorphic (*Aat-3*B; *Gdh*-A; *Pgm*-B). The remaining 21 polymorphic loci (44%) appear in Table 2.

Distribution and correlation of alleles with the microclimatic niches: Several loci varied significantly be-

Table 1. Temperature and relative humidity in sun and in shade at Yehudiyya, measured on May 8, 1987 (N= 10 trees)

Microniche		Sun		Shade		Sun-shade differential	
		Mean	SE	Mean	SE	Mean	SE
Temperature:	Air	32.45	±0.617	30.35	±0.269	2.10*	±0.515
	Soil	39.35	±0.943	29.37	±0.440	9.98*	±0.922
	Soil-air differential	6.90*	±0.833	−0.98*	±0.289		
Relative humidity:	Air	36.95	±1.184	37.6	±1.213	−0.65 ns	±0.460
	Soil	37.30	±1.265	37.7	±1.274	−0.40 ns	±0.476
	Soil-air differential	0.35 ns	±0.279	0.1 ns	±0.277		

Significance (t-test): * = $P < 0.001$; ns = $P < 0.10$; air temperature taken 1 m above ground

Table 2. Allele frequencies at 21 polymorphic loci of 4 subpopulations of *Triticum dicoccoides* (sunny and shady microniches in two years) at Yehudiyya, Israel

Year			1984		1985			χ^2 between sun and shade (1 df)
Microniche			Sun	Shade	Sun	Shade	Mean	
Size (N)			14	16	60	47	137	
Locus	+ − Allele	Compl. allele						
Aat-2A	a − +	(b)	0.083	0.393	0.136	0.128	0.155	0.427 ns
	He		0.153	0.477	0.234	0.223	0.272	
Aat-3A	a − −	(null)	0.917	1.000	0.932	1.000	0.962	Fisher @
	He		0.153	0.0	0.126	0.0	0.070	
Acph-3	b − +	(c)	0.167	0.727	0.583	0.553	0.546	0.417 ns
	He		0.278	0.397	0.486	0.494	0.414	
Acph-X	a −	(b)	0.0	0.700	− − −	− − −	0.389	Fisher**
	He		0.0	0.420	− − −	− − −	0.210	
Adh-1A	a −	(b)	1.000	1.000	0.310	0.529	0.500	2.656 ns
	He		0.0	0.0	0.428	0.498	0.232	
Adh-2A	a −	(b)	1.000	1.000	0.817	0.979	0.902	5.980*
	He		0.0	0.0	0.299	0.042	0.085	
Est-1	b +	(c)	0.429	0.409	− − −	− − −	0.417	Fisher ns
	Null −		0.429	0.455	− − −	− − −	0.444	Fisher ns
	He		0.612	0.607	− − −	− − −	0.610	
Est-2A	b +	(c)	0.700	0.0	− − −	− − −	0.500	Fisher @
	He		0.420	0.0	− − −	− − −	0.210	
Est-2B	c −	(a)	0.200	1.000	− − −	− − −	0.429	Fisher @
(a = null)	d +		0.700	0.0	− − −	− − −	0.500	Fisher*
	He		0.460	0.0	− − −	− − −	0.230	
Est-4B	c −	(null)	0.400	0.600	1.000	1.000	0.727	0.166 ns
	He		0.480	0.480	0.0	0.0	0.240	
Est-5A	a −	(d)	0.167	0.667	1.000	1.000	0.885	0.569 ns
	He		0.278	0.444	0.0	0.0	0.181	
Est-5B	a + −	(c)	0.833	0.533	0.136	0.233	0.279	2.766 ns
(a = null)	e − +		0.167	0.467	0.814	0.628	0.651	
	He		0.278	0.498	0.317	0.532	0.406	
Gluc-A	a +	(null)	0.778	0.625	0.0	0.0	0.097	0.0 ns
	He		0.346	0.469	0.0	0.0	0.204	
Gluc-B	a −	(b)	0.333	0.875	1.000	1.000	0.944	Fisher ns
	He		0.444	0.219	0.0	0.0	0.166	
Hk	a +	(b)	0.714	0.100	− − −	− − −	0.353	Fisher*
	He		0.408	0.180	− − −	− − −	0.294	
Ipol	b −	(c)	0.500	0.643	1.000	1.000	0.954	Fisher ns
	He		0.500	0.459	0.0	0.0	0.240	
Mdh-1A	a −	(b)	0.308	0.667	1.000	1.000	0.896	0.277 ns
	He		0.426	0.444	0.0	0.0	0.218	
Pept-1B	a − −	(b)	0.300	0.714	0.783	0.864	0.766	1.682 ns
	He		0.420	0.408	0.339	0.236	0.351	
Pept-3	b + −	(c)	0.700	0.385	0.061	0.171	0.196	0.279 ns
	He		0.420	0.473	0.115	0.284	0.323	
Pgi-A	a + +	(b)	0.846	0.615	0.300	0.255	0.368	0.336 ns
	He		0.260	0.473	0.420	0.380	0.383	
6Pgd-2	a + +	(c)	0.818	0.429	0.515	0.381	0.506	2.130 ns
	He		0.298	0.490	0.500	0.472	0.440	

Monomorphic loci: Aat-1A, 1B, 2B, Adh-1B, 2B, Cat-A, B, Est-4A, Gdh-B, Ipor-A, B, Mdh-1B, 2, Nadh-1A, 1B, 2, Pepc, Pept-1A, 2, Pgi-B, Pgm-A, 6Pgd-1A, 1B
Low polymorphic loci: Aat-3B, Gdh-A, Pgm-B
Abbreviations: Compl. = Complementary; + = Higher frequency in sun; − = Higher frequency in shade; − − − = Missing data
Fisher = Fisher exact test
Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; @ = $P < 0.10$; ns = $P > 0.10$

Table 3. Summary of genetic variation based on 48 loci of *Triticum dicoccoides* in two microclimatic niches and two years at Yehudiyya

Locality	Sample size (*N*)	Mean no. of alleles per locus (A)	Mean proportion of loci				Genetic diversity (He)
			Polymorphic per population (P)		Heterozygous per individual (H)		
			(1%)	(5%)	Mean	SE	
1984							
Sunny niche	14	1.426	0.383	0.383	0.0	0.0	0.141
Shady niche	16	1.383	0.362	0.362	0.006	0.004	0.150
1985							
Sunny niche	60	1.302	0.233	0.233	0.001	0.001	0.077
Shady niche	47	1.233	0.209	0.186	0.0	0.0	0.074
Mean	137	1.336	0.297	0.291	0.002	0.001	0.110
Summarized over 1984 + 1985							
Sunny niche	74	1.521	0.417	0.396	0.001	0.000	0.145
Shady niche	63	1.438	0.396	0.313	0.003	0.002	0.116
Mean	137	1.479	0.406	0.354	0.002	0.001	0.131

Table 4. Partition of genetic diversity of *Triticum dicoccoides* in microclimatic niches at Yehudiyya, based on 16 polymorphic loci (*Gst* analysis; Nei 1973)

Locus	Alleles	Sample	*Ht*	*Hs*	Dst@			*Gst*	*Dm*	*Rst*
					Total	Sun-shade	%			
Aat-2A	2	132	0.2624	0.2486	0.0138	0.0019	13.8%	0.0526	0.0184	0.0740
Aat-3A	2	132	0.0729	0.0704	0.0025	0.0025	98.5%	0.0343	0.0033	0.0474
Acph-3	2	130	0.4957	0.4623	0.0334	0.0026	7.8%	0.0675	0.0446	0.0965
Adh-1A	2	56	0.5000	0.3729	0.1271	0.0323	25.4%	0.2541	0.1694	0.4543
Adh-2A	2	122	0.1774	0.1633	0.0141	0.0110	78.0%	0.0793	0.0187	0.1148
Est-4B	2	44	0.3967	0.2727	0.1240	0.0051	4.1%	0.3125	0.1653	0.6061
Est-5A	2	131	0.2028	0.0763	0.1264	0.0016	1.3%	0.6236	0.1686	2.2087
Est-5B	3	129	0.4932	0.4062	0.0871	0.0052	6.0%	0.1765	0.1161	0.2858
Gluc-A	2	124	0.1748	0.0553	0.1195	0.0001	0.1%	0.6835	0.1593	2.8793
Gluc-B	2	124	0.1065	0.0464	0.0602	0.0023	3.8%	0.5647	0.0802	1.7298
Ipol	2	120	0.0875	0.0518	0.0357	0.0000	0%	0.4079	0.0476	0.9186
Mdh-1A	2	135	0.1859	0.0904	0.0955	0.0009	0.9%	0.5137	0.1273	1.4083
Pept-1B	2	128	0.3589	0.3175	0.0414	0.0064	15.5%	0.1152	0.0551	0.1736
Pept-3	2	107	0.3155	0.2423	0.0732	0.0018	2.5%	0.2319	0.0975	0.4025
Pgi-A	2	133	0.4654	0.3956	0.0698	0.0020	2.9%	0.1500	0.0931	0.2353
6Pgd-2	2	79	0.4999	0.4623	0.0376	0.0180	47.9%	0.0753	0.0502	0.1086
Mean	2.06	114.1	0.2997	0.2334	0.0663	0.0059	8.9%	0.2212	0.0884	0.3787

Abbreviations: *Ht* = Total gene diversity; *Hs* = Average gene diversity within populations; *Dst* = Average gene diversity between populations; *Dm* = Average of interpopulational diversity only; *Gst* = Gene diversity between populations, relative to *Ht*; *Rst* = Interpopulational diversity, relative to *Hs*; @ = % of sun-shade *Dst* from total *Dst* which includes also the genetic difference between years

tween the sun and shade microniches (Table 2). Consistent association between allozymes and microclimatic niches was found in four loci (*6Pgd-2, Pgi*-A, *Aat-3*A and *Pept-1*B) and alleles, first with sun (*6Pgd-1*^{a}, *Aat-3*A^{null}, *Pgi*-A^{a}, *Pept-1*B^{b}) and then with shade (*6Pgd-2*^{c}, *Aat-3*A^{a}, *Pgi*-A^{b}, *Pept-1*B^{a}). Interestingly, the *Aat-3*A^{null}, allele appeared only in the sunny microniche. Five polymorphic loci (*Est-1, Est-2*A, 2B, *Acph-X, Hk*) were not scorable in 1985, and therefore were not included in this analysis or in the following multilocus analyses.

Genetic summary. The genetic summary of the two climatic microniches in each year appear in Table 3. The estimates of gene diversity *He,* (Nei 1975), do not vary significantly between the two microniches. However, the levels of polymorphism, *P,* and number of alleles per locus, *A,* were higher in sun than in shade in both years.

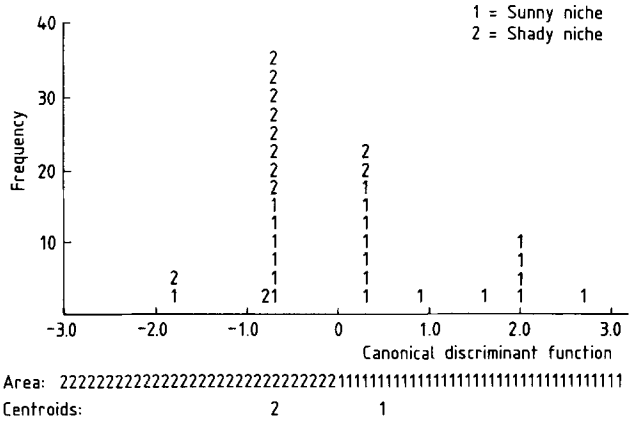

Fig. 1. Discriminant analysis of wild emmer wheat in Yehudiyya according to two climatic microniches: sunny and shady, based on seven polymorphic isozyme loci

Table 5. Stepwise discriminant analysis of genetic diversity of *Triticum dicoccoides* between sunny and shady microniches in Yehudiyya. Pairwise comparison between sunny and shady microniches. The F statistic has 7 and 74 degrees of freedom

	Sun
Shade	F = 3.616 $P < 0.0021$

$N = 82$ (out of 137); 50 on sunny, 32 on shady microniches

Table 6. Summary Table of discriminant analysis (Chosen variables)

Step	Action	Variables in	Wilks' Lambda	Significance
	Entered-Removed			
1	Adh-2A b	1	0.90085	0.0040
2	Aat-3A null	2	0.84485	0.0013
3	Pgi-A a	3	0.82490	0.0017
4	Mdh-1A b	4	0.80420	0.0019
5	Est-5B e	5	0.78388	0.0020
6	Aat-2A a	6	0.76601	0.0022
7	Pept-3 b	7	0.74511	0.0021

Analysis of genetic diversity, within and between populations. Gene diversity of a subdivided population can be analyzed into its components, i.e., the gene diversities within and between subpopulations. The results provide measures of the average (*Dst*) absolute (*Dm*), relative (*Gst*) and inter- versus intrapopulational (*Rst*) degree of gene differentiation among subpopulations where *Hs* is the mean gene diversity within a population, *Ht* in the total population and $Ht = Hs + Dst$ (Nei 1973). *Dm* can be used to compare the degrees of gene differentiation in different organisms.

Considering each of the two climatic microniches as subpopulations (Table 2), the *Dst* analysis of gene diversity of the 16 polymorphic loci is given in Table 4. On the average, 77.9% of the total genetic diversity in the subpopulations (2 microniches and 2 years) exists within, and 22.1% exists between subpopulations. These results indicate a relatively strong gene differentiation between subpopulations. The contrasting climatic microniches contributed 8.9% to the genetic differentiation, but in certain loci (*Aat-3*A. *Adh-1*A, *Adh-2*A, *6Pgd-2*) the microclimate contribution was greater. Thus, allozyme differentiation between the two climatic microniches is substantive, as is also displayed in the following discriminant analysis.

Discriminant analysis. We conducted stepwise discriminant analysis (SPSS-x 1986), maximizing the overall multivariate F ratio of the two climatic microniches, based on a multilocus analysis involving 12 polymorphic loci and 14 alleles, from which the program chose 7 alleles as the best differentiating factors (*Adh-2*A^{b}, *Aat-3*A^{null}, *Pgi-*A^{a}, *Mdh-1*A^{b}, *Est-5*B^{e}, *Aat-2*A^{a}, *Pept-3*^{b}; Table 6). We selected the preliminary 12 out of the 21 polymorphic loci tested, in order to minimize the reduction of sample size for this analysis due to missing data. The results are given in Fig. 1 and Tables 5–8.

The analysis succeeded to differentiate significantly between plants from the two climatic microniches by pairwise comparison ($F = 3.62$; d.f. 7/74; $P < 0.01$; Table 5) and by a canonical discriminant function (Table 7 and Fig. 1). The correct classification of plants into their two climatic microniches was 73% (62 out of 85 analyzed plants): 68% in the sunny and 80% in the shady microniche (Table 8).

Genetic distances. Coefficients of genetic distances (*D*), and genetic similarity (*I*), were calculated to compare

Table 7. Canonical discriminant functions

Function	Eigenvalue	Canonical correlation	After function	Wilks' lambda	Chi-squared	df	Significance
1	0.34207	0.5049	0	0.7451	22.508	7	0.0021

Table 8. Classification results of discriminant analysis

Actual group	No. of cases	Predicted group membership	
		1	2
Sunny niche	50	34 68.0%	16 32.0%
Shady niche	35	7 20.0%	28 80.0%
72.94% of plants correctly classified			

Table 9. Coefficients of genetic similarity (*I*, upper figure), and genetic distance (*D*, middle figure) with standard error (lower figure) between four subpopulations of *Triticum dicoccoides* in Yehudiyya

Population	2	3	4
1984			
Sun	0.909 0.096 0.012	0.824 0.194 0.012	0.833 0.183 0.012
Shade		0.893 0.114 0.008	0.902 0.103 0.008
1985			
Sun			0.996 0.004 0.002
Shade			
I: Mean 0.893	Range 0.824–0.996		
D: Mean 0.115	Range 0.004–0.194		

the four subpopulations, based on the normalized identity of all loci between each pair of subpopulations (Nei 1972). The results are given in Table 9. The mean value of *D* was 0.115, range 0.004–0.194. All standard errors of individual values of *D* were less than 0.013. The estimates of *D* indicated sharp genetic differentiation between the two climatic microniches ($D=0.05$, range 0.004–0.096).

Genetic differentiation at the two-locus level

Gametic phase disequilibria (D) for the entire sample and for each climatic microniche (sunny and shady) are given in Table 10. D's can be classified into general (occurring in both sun and shade) and *specific* (occurring either in sun or in shade). Examples of general D's were: *Mdh-1*A^a – *Est-4*B^c ($D=0.185$, 0.152 in sun and shade, respectively); *Pept-3*^{b} – *Pept-1*B^b ($D=0.112$, 0.142, respectively). Specific D's for the sunny microclimatic niche were: *Pgi-*A^b – *Adh-2*A^a ($D=0.111$); *Pept-1*B^b – *Adh-2*A^a ($D=0.120$). Specific D's for the shady microclimatic niche were: *6Pgd-2*^{c} – *Est-4*B^c ($D=0.228$).

Multilocus organization; sun versus shade

Multilocus organization index, related to the single-locus Simpson index, and based on the observed distribution of the number of heterozygous loci (K) in two randomly chosen gametes, was proposed by Brown et al. (1980). It measures multilocus associations when multiple alleles and many loci are analyzed, combining all paired-loci gametic-phase-disequilibria. We calculated multilocus orgnaization indices for the entire data set, and for the sunny and shady microniches in this study, separately. The results are given in Table 11. The differences between the expected and observed variances (S_K^2) are significant in each microniche, and the observed variance of K in the entire data set is 248% above the expected variance. Multilocus organization appears to be significantly higher in the sunny microclimatic niche than in the shady one. The standardized index of intensity of multilocus structure X(2) was 3.0 in the sun and 2.3 in the shade. We conclude that on average, multilocus organization is higher in the sunny microniche.

Discussion

We demonstrated earlier single and multilocus genetic differentiation in wild emmer wheat at both the macro- and microgeographical scales (Nevo et al. 1982a; Golenberg 1986; Golenberg and Nevo 1987). In previous analyses, we analyzed environmental effects combining both climate and soil. Recently, we attempted to separate soil and microclimatic effects in our complementary paper (Nevo et al. 1988), although we realize that the aridity index appears to play a predominant role either indirectly (i.e. through different soil types) or directly. Here, we demonstrated that alozyme differentiation is sensitive not only to macro- but, most importantly, to direct microclimatic factors. First, we will briefly discuss the microclimatic contrasting factors related to our previous work in wild barley (Nevo et al. 1986). We will conclude by discussing population genetic differentiation in wild emmer and their ecological determinants.

Shade ecology

The climatic profile of open shade as a microclimatic niche was analyzed in Europe by Stoutjesdijk (1974). While different types of open shade may differ in their climatic profiles, the major climatic determinants of the open shade i.e., temperature, humidity and radiation

Table 10. Representative gametic phase disequilibria (D) between paired loci of *Triticum dicoccoides* in Yehudiyya; in the entire sample, and separately for each climatic microniche

Locus and allele	Locus and allele	Over all *P*	Sun *P*	Shade *P*
Mdh-1A a	Est-4B c	0.171***	0.185** (c)	0.152*** (c)
Mdh-1A a	Est-5A a	0.088***	0.109*** (c)	0.062*** (c)
Est-5A a	Est-4B c	0.152***	0.166** (c)	0.123***
Pept-1B a	Est-4B c	0.123**	0.133* (c)	0.086*
Pept-3 b	Pept-1B b	0.123***	0.112*** (c)	0.142***
Pept-3 c	Est-5A a	0.084* (c)	0.101*** (c)	0.065** (c)
Pept-3 c	Est-4B c	0.185*** (c)	0.240*** (a)	0.136** (c)
Pept-1B b	Pgi-A a	0.120***	0.161***	0.064* (c)
Pept-3 b	Gluc-A a	0.076***	0.100*** (c)	0.043 NS
Pept-1B b	Gluc-A a	0.059***	0.072*** (c)	0.041@
Gluc-A null	Est-4B c	0.163***	0.245*** (a)	0.090@
Gluc-B a	Est-4B c	0.123*** (c)	0.204** (c)	0.040 NS (c)
Ipol b	Est-4B c	0.098**	0.168** (a)	0.045 NS
Pgi-A b	Adh-2A a	0.070*** (c)	0.111*** (c)	0.014 NS (c)
Pept-1B a	Adh-2A a	0.071***	0.120***	–
6Pgd-2 a	Adh-1A a	0.071 NS (c)	0.141* (c)	–
6Pgd-2 a	Adh-2A a	0.059** (c)	0.124*** (c)	–
6Pgd-2 a	Pgi-A a	−0.009 NS	−0.087*	0.074@
6Pgd-2 c	Est-4B c	0.183*** (c)	0.118 NS (c)	0.228*** (a)
Acph-X a	Est-4B c	0.188** (c)	–	0.148@ (c)
Aat-2A a	Adh-1A a	0.030 NS	−0.001 NS	0.068 NS (c)
Aat-2A a	Est-4B c	0.032 NS (c)	–	0.047 NS (c)
Acph-3 b	Adh-1A a	0.063@	0.016 NS	0.112@

Significance: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; @ = $P<0.10$; NS = $P>0.10$; *P* by chi square with Yates correction, or by Fisher's exact test
(c) = complete association: one gametic type is missing (Clegg et al. 1976)
(a) = absolute association: only two gametic types are present (Clegg et al. 1976)

Table 11. Estimates of multilocus genetic organization of *Triticum dicoccoides* in two climatic microniches in Yehudiyya

Microniche	Polymorphic loci	Expected var of K	Upper Limit of 95%	Estimate of var of K[a]	X(2)[b]
Sun	16	3.142	< 5.553	12.571*	3.001
Shade	15	2.574	< 4.295	8.543*	2.319
Sun + shade	16	2.948	< 4.464	10.262*	2.481

Significance: * $P<0.05$
[a] K = number of heterozygous loci in two randomly chosen gametes
[b] X(2) = measure of multilocus structure

conditions, may display parallel patterns. Surface temperatures are often 6°–8° below ambient temperatures, humidity is higher than that in the sunny microniche, and dew may persist in the open shade. Light intensity is relatively high, but net radiation may be zero or slightly negative due to the strong long-wave radiation loss to the "cold" bright sky. The specific vegetation in open shade sites reflects habitat conditions. Several angiosperms, mosses and liverworts prefer this microniche. While the under oak canopy in our study area at Yehudiyya is not an open shade but rather a covered shade, it appears to share several microclimatic characteristics with the open shade. These appear to involve more relatively constant temperature and humidity conditions in the shady microniche as compared to the sunny microniche. In the covered shade, temperature is lower and humidity slightly higher (Table 1).

Microclimatic analysis of allozyme differentiation in wild barley

The oak under-canopy shade conditions comprise a unique microclimatic niche affecting genetic differentiation, as we have previously demonstrated in wild barley *Hordeum spontaneum* (Nevo et al. 1986). The latter test involved six microniches at the Neve Yaar microsite in Israel, organized in a mosaic pattern in the open Tabor oak forest. The microniches were (1) sun soil. (2) sun rock, (3) shade soil and (4) shade rock and two contact zones. The two contact zones were (5) soil periphery of the sun-rock microniche and (6) soil periphery of the shade-rock microniche. Discriminant analysis indicated significant multilocus allozymic differentiation between the microniches: the stressful sun rock differed significantly from the shade-rock microniche, as did the soil contacts of shade rock and sun rock with the other microniches.

All in all, in the wild barley microsite mossaic study at Neve Yaar, we have shown genetic differentiation over very short distances associated with the ecological patchiness of the environment. Genetic diversity proved highest in the sun-rock microniche, which is the driest at the end of the growing season. Therefore, this sun-rock microniche displays a wider ecological variance over time, in accordance with the prediction of the niche width-variation hypothesis (Van Valen 1965), which predicts a positive correlation between ecological and genetic diversities.

Neighbor effects, population size, and migration in wild emmer

It is important, however, to avoid the pitfall of neighboring effect (Jain 1976) in the attempt to separate random (founder effect) from nonrandom (niche structure) factors. Our present data at least partly overcomes this problem, since ours was a series of repetitive experiments, each consisting of an oak tree undercanopy and its surrounding intertree space. The mosaic oak tree structure of our experimental design, plus the 18 repetitions in both years, safeguard, at least partly, from genetic neighborhood effects. The latter was estimated to be about 5 m in diameter in Yehudiyya (Golenberg 1986). Furthermore, neither small-size population effects nor migration patterns can singly or in combination explain the pattern discovered. Population size of wild emmer in Yehudiyya is big, involving many thousands of individuals. Migration role in this population was estimated to be low, about 1.25 m per generation (Golenberg 1986). While this appears to be a relatively low migration rate, it suffices to equalize allele frequencies during relatively short periods. The sun-shade genetic differentiation found is therefore unlikely to be the result of stochastic processes. Rather, it appears to reflect microclimatic niche differentiation in a relatively small area of less than 1,000 m^2, and over few meters between sun and shade.

The Yehudiyya-Qazrin genetic differentiation of wild emmer

Significant differences were found among populations of the Yehudiyya and nearby (10 km apart) Qazrin genotypes for morphological, germination and phenological characters when grown under standardized greenhouse conditions (Golenberg 1986). Reciprocal transplantation and replacement series competition experiments did not, however, reveal any local adaptations in the short periods of testing, possibly because of the short duration of the experiment and technical disturbances. Transplant experiments with the shade-sun allozyme genotypes of Yehudiyya, involving both standardized greenhouse and field experiments, should be very rewarding to assess the differential fitness of the allozyme genotypes in their own and alternative microclimatic niches.

Multilocus microclimatic differentiation in wild emmer

Present results at the Yehudiyya site of genetic differentiation, according to the microclimatic niche, suggest that genetic diversity and allele frequency differentiation at the single level, but primarily at the multilocus levels, are subject to selection. This is indicated by: (1) single loci differentiation (Table 2), but *primarily* by multilocus discrimination (Tables 5–8); (2) higher levels of polymorphism in sun in contrast to shade, according to the prediction of the niche width-variation hypothesis; (3) microclimatic specificity of two-locus gametic phase disequilibria for sun as opposed to shade (Table 10); and (4) multilocus genome organization (Table 11). At the multilocus level, there is substantial multilocus organization. On average, the variance of K was 248% higher than expected under random association. Interestingly, the sunny multilocus genotype showed more multilocus organization than that of the shady one (300% vs 232%, respectively. Table 11). It is worth noting that genetic diversity, *He,* based on our regional analysis (Nevo et al. 1982a), is also associated with higher ecological unpredictability in climatically fluctuating steppe-like regions (Nevo and Beiles 1988).

Aridity stress selection in wild emmer and other organisms

The present sun-shade microniche study at the Yehudiyya microsite supports earlier studies, indicating sharp microgeographical differentiation in other plants such as wild oats *Avena barbata* (Hamrick and Allard 1972; Hamrick and Holden 1979), and *Litaris cylindracea* (Schaal 1974, 1975). (For reviews on microgeographical genetic differentiation see Brown 1979 and Nevo 1983 a). The effects of stress as an agent in unravelling the operation of natural selection was demonstrated earlier in microsite differentiation of allozymes in barnacles (Nevo et al. 1977), in landsnails (Nevo et al. 1982b), in wild barley, *Hordeum spontaneum* (Nevo et al. 1981, 1983, 1986) and in wild emmer (Nevo et al. 1988). The effects of stress on allozyme differentiation were also demonstrated in controlled laboratory experiments of pollution biology (reviewed in Nevo 1986).

Aridity stress was previously implicated in allozyme differentiation of many unrelated species in Israel (Nevo 1983c), and worldwide (Nevo et al. 1984). In a recent analysis of genetic parallelism in genetic diversity of 21 species in Israel, we concluded that natural

selection may have operated directly, at least on some of the allozyme loci studied, rather than indirectly, through the hitchhiking effect (reviewed by Hedrick 1982). In the present study, we have no direct evidence implicating ecological selection of specific allozymes as opposed to linked blocks of genes. However, our evidence supports the hypothesis that genetic differentiation at the Yehudiyya microsite is affected, at least partly, by the aridity stress faced by various life cycles of wild emmer.

Our present data are inconsistent with the hypothesis that allozymic diversity is neutral, and that its fate is determined by mutation input and random fixation, as suggested by the neutral theory of molecular evolution (Kimura 1983). Allozyme differentiation, particularly at the multilocus level as the unit of selection, corresponds to the two microclimatic niches. The aridity index presumably plays a major differentiating role here and elsewhere (Nevo et al. 1981, 1982a, b, 1983, 1986; Nevo et al. 1988). The higher levels of polymorphism in the sunny microniche may be regarded as an adaptive strategy for increasing fitness in a more climatically fluctuating microniche, in accordance with the niche variation hypothesis (Van Valen 1965). This result supports regional (Nevo 1983c; Nevo and Beiles 1988) and global (Nevo et al. 1984) patterns, where allozymic diversity generally increases with increasing spatiotemporal ecological heterogeneity and unpredictability. Our results support the theoretical prediction that the existence of a protected polymorphism is more likely in a more heterogeneous environment (e.g. Karlin 1981, 1982). We hypothesize that the patterns observed here are, to a large extent, due to a diversifying balancing selection on allozyme polymorphism. Fitness tests through transplant experiments, and biochemical tests of alternative allozyme alleles, should follow to verify the correlative results presented here.

Our results implicate not only theoretical, but more importantly, practical applications. Since allozymic diversity varies dynamically in space and time, at both the macro- and microgeographical levels, with varying and/or changing ecologies, sampling strategies should be designed accordingly. If macro- and microecological differentiation are closely followed, sampling strategies, exploitation, conservation, and utilization of wild emmer for breeding could be substantially maximized (Nevo 1983b; Brown and Clegg 1983).

Acknowledgements. We thank E. M. Golenberg for field work and for commenting on the manuscript. This research was supported by the Wolfson Foundation, the Israel Discount Bank Chair of Evolutionary Biology, the 'Ancell-Teicher Research Foundation for Genetics and Molecular Evolution' established by Florence and Theodore Baumritter of New York, and by Humana Inc., Kentucky.

References

Allard RW, Babbel GR, Clegg MT, Kahler AL (1972) Evidence for coadaptation in *Avena barbata.* Proc Natl Acad Sci USA 69:3043–3048

Brown AHD (1979) Enzyme polymorphism in plant populations. Theor Popul Biol 15:1–42

Brown AHD, Clegg MT (1983) Isozyme assessment of plant genetic resources. In: Rattazzi MC, Scandalios IG, Whitt GS (eds) Isozymes: Current topics in biological and medical research, vol 11. Medical and other applications. A. R. Liss. New York, pp 285–295

Brown AHD, Feldman MW, Nevo E (1980) Multilocus structures of natural populations of *Hordeum spontaneum.* Genetics 96:523–536

Clegg MT, Kidwell JF, Kidwell MG, Daniel NJ (1976) Dynamics of correlated genetic system. I. Selection in the region of the Glued locus of *Drosophila melanogaster.* Genetics 83:793–810

Dan J, Nissim S (1972) The soils of the Middle Golan. Preliminary report. Volcani Inst Agric Res Div Sci Publ Pam, Bet Dagan, Israel

Feldman M (1976) Wheats. In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 120–128

Golenberg EM (1986) Multilocus structures in plant populations: Population and genetic dynamics of *Triticum dicoccoides.* Ph D thesis. State University of New York at Stony Brook, New York

Golenberg EM, Nevo E (1987) Multilocus differentiation and population structure in a selfer, wild emmer wheat. *Triticum dicoccoides.* Heredity 58:451–456

Hamrick JL, Allard RW (1972) Microgeographical variation in allozyme frequencies in *Avena barbata.* Proc Natl Acad Sci USA 69:2100–2104

Hamrick JL, Holden LR (1979) Influence of microhabitat heterogeneity on gene frequency distribution and gametic phase disequilibrium in *Avena barbata.* Evolution 33: 521–533

Hedrick PW (1982) Genetic hitchhiking: A new factor in evolution. Bio Science 32:845–853

Jain SK (1976) Patterns of survival and microevolution in plant populations. In: Karlin S and Nevo E (eds) Population genetics and ecology. Academic Press, New York, pp 49–75

Karlin S (1981) Some natural viability systems for multiallelic locus: A theoretical study. Genetics 97:457–473

Karlin S (1982) Classifications of selection migration structures and conditions for a protected polymorphism. Evol Biol 14:61–204

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Nei M (1975) Molecular population genetics and evolution. Elsevier/North Holland, Amsterdam

Nevo E (1983a) Adaptive significance of protein variation. In: Oxford GS, Rollingson D (eds) Protein polymorphism: adaptive and taxonomic significance, vol 24. Systematic Association Special. Academic Press. New York, pp 239–282

Nevo E (1983b) Genetic resources of wild emmer wheat: Structure, evolution and application in breeding. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto University, Kyoto, Japan, pp 421–431

Nevo E (1983c) Population genetics and ecology: The interface. In: Bendall DS (ed) Evolution from molecules to men. Cambridge University Press. England, pp 287–321

Nevo E (1986) Pollution and genetic evolution of marine organisms: Theory and practice. In: Dubinsky Z, Steinberger Y (eds) Environmental Quality and ecosystem stability, vol III A/B. Bar-Ilan University Press. Ramat Gan. Israel, pp 841–848

Nevo E, Beiles A (1988) Genetic parallelism of protein polymorphism in nature: Ecological test of the natural theory of molecular evolution. (submitted)

Nevo E, Beiles A, Ben-Shlomo R (1984) The evolutionary significance of genetic diversity: Ecological, demographic and life history correlates. In: Mani GS (ed) Evolutionary dynamics of genetic diversity, vol 53. Lecture notes in biomathematics. Springer, Berlin Heidelberg New York, pp 13–213

Nevo E, Beiles A, Krugman T (1988) Natural selection of allozyme polymorphisms: A microgeographical differentiation by edaphic, topographical and temporal factors in wild emmer wheat (*Triticum dicoccoides*). (submitted)

Nevo E, Shimony TM, Libni M (1977) Thermal selection of allozyme polymorphisms in barnacles. Nature 267:699–701

Nevo E, Bar-El C, Beiles A, Yom-Tov Y (1982b) Adaptive microgeographic differentiation of allozyme polymorphisms in landsnails. Genetica 59:61–67

Nevo E, Beiles A, Kaplan D, Golenberg EM, Olsvig-Whittaker, Naveh Z (1986) Natural selection of allozyme polymorphisms: A microsite test revealing ecological genetic differentiation. Evolution 40:13–20

Nevo E, Beiles A, Storch N, Doll H, Anderson B (1983) Microgeographic edaphic differentiation in hordein polymorphisms of wild barley. Theor Appl Genet 64:123–132

Nevo E, Brown AHD, Zohary D, Storch N, Beiles A (1981) Microgeographic edaphic differentiation of allozyme polymorphism of wild barely. Plant Syst Evol 138:287–292

Nevo E, Golenberg EM, Beiles A, Brown AHD, Zohary D (1982a) Genetic diversity and environmental associations of wild wheat *Triticum dicoccoides* in Israel. Theor Appl Genet 62:241–254

Sas (1985) User's guide, 5th edn. SAS Institute, Cary, North Carolina

Schall BA (1974) Isolation by distance in *Liatris cylindracea.* Nature 252:703

Schall BA (1975) Population structure and local differentiation in *Liatris cylindricea.* Am Nat 108:511–528

SPSS-x (1986) User's guide, 2nd edn. McGraw Hill, New York

Stoutjesdijk P (1974) The open shade, an interesting microclimate. Acta Bot Neeri 23:125–130

Van Valen L (1965) Morphological variation and width of ecological niche. Am Nat 99:377–390

Zohary D (1970) Centers of diversity and centers of origin. In: Frankel OH, Bennet E (eds) Genetic resources in Plants – their exploration and conservation. Blackwell Oxford, pp 33–42

Zohary M (1973) Geobotanical foundations to the Middle East, vols 1 and 2. Fischer, Stuttgart/Swets and Zeitlinger, Amsterdam