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Natural selection of allozyme polymorphisms: a microgeographical differentiation by edaphic, topographical, and temporal factors in wild emmer wheat (*Triticum dicoccoides*)

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Summary. Allozymic variation in proteins encoded by 47 loci was analyzed electrophoretically in 1983/4 and 1984/5 in 356 individual plants of wild emmer wheat, *Triticum dicoccoides*, from a microsite at Tabigha, north of the Sea of Galilee, Israel. Each year the test involved two 100-meter transects, each equally subdivided into basalt and terra rossa soil types, and comparisons were based on 16 common polymorphic loci. Significant genetic differentiation, genetic phase disequilibria, and genome organization according to soil type were found over very short distances. Our results suggest that allozyme polymorphisms in wild emmer wheat are partly adaptive, and that they differentiate at both single and multilocus structures primarily from environmental stress of such ecological factors as soil type, topography, and temporal changes, probably through aridity stress.

Key words: Genetic diversity – ecological selection – microgeographic differences – *Triticum dicoccoides*

Introduction

The essence and evolutionary dynamics of allozyme polymorphisms in nature are still controversial. Present evidence, based on global (Nevo 1978; Hamrick et al. 1979; Nevo et al. 1984), regional (e.g., Nevo et al. 1982a; Nevo 1983a; Heywood and Levin 1985), and local (e.g., Allard et al. 1972; Hamrick and Allard 1972; Schaal 1974, 1975; Baker et al. 1975; Nevo et al. 1977; Brown 1979; Hamrick and Holden 1979; Nevo et al. 1981, 1988) analyses supports natural selection as the major genetic force behind the differentiation of allozymes in populations and species. Nevertheless, the neutral theory suggests that the molecular evolution of proteins and DNA is largely nonselective, or neutral, being maintained in populations through mutational input and random fixation (Kimura 1983).

Microgeographical studies of allozyme differentiation could contribute to a better understanding of the nature and dynamics of allozyme polymorphisms (e.g. for plants, see Brown 1979; for animals, Selander and Kaufman 1975). In some cases, such as in the landsnail *Helix aspersa*, the microgeographic differentiation of allozymes cannot resolve the question of whether selection or drift cause genetic differentiation (Selander and Kaufman 1975). By contrast, in other cases, such as in *Drosophila*, significant microspatial differentiation in chromosome inversion and allozyme frequencies are prevalent (Richmond 1978). Additional critical tests are therefore called for.

During the last decade we have conducted extensive studies on allozyme differentiation in many species of plants and animals in Israel, both at the macro- (reviewed in Nevo 1983a) and microgeographic levels (Nevo et al. 1977; Nevo et al. 1981, 1982b, 1983, 1986). These studies suggest that allozyme polymorphisms are at least partly adaptive. Our microsite studies indicate that microniche ecological selection, rather than stochastic processes and/or the neutrality of allozymic variants, is the primary cause of genetic differentiation in barnacles, landsnails, and wild barley.

The objective of the present study was to test the hypothesis that edaphic natural selection plays an important role in allozyme differentiation of wild emmer wheat, *Triticum dicoccoides*. We repeated tests with wild emmer wheat which were similar in their experimental design to those conducted earlier with wild barley, *Hordeum spontaneum* (Nevo et al. 1981, 1983). Data

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from a parallel critical experiment in wild emmer suggests once again that the selective force exerted by natural selection at both the single and multilocus levels is substantial in the genetic differentiation of a local edaphically, topographically and temporally differentiated population of wild emmer.

Materials and methods

Wild emmer wheat

Wild emmer, *T. dicoccoides*, (referred to as *T. turgidum* var 'dicoccoides' in Kimber and Feldman 1987) (genomic constitution AABB) is the allotetraploid wild progenitor of all bread wheats (Zohary 1970; Feldman 1976). It is distributed over the Near East Fertile Crescent, but its center of distribution is found in the Upper Jordan Valley and surroundings. In this area, wild emmer grows as a highly selfing, annual grass, in several steppe-like herbaceous formations and in the open oak park forest belts of *Quercus ithaburensis* or *Q. brantii* (Zohary 1973). It grows mainly on basaltic and terra rossa soil types and is restricted chiefly 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 (e.g., in our Tabigha microsite), as low as 100 m below sea level. More slender and late-flowering types occur at higher elevations reaching 1400 m on Mount Hermon (Zohary 1970). The population genetics of wild emmer, based on 50 electrophoretically tested gene loci has been described by Nevo et al. (1982a), and the multidisciplinary studies conducted on wild emmer at the Institute of Evolution have been reviewed by Nevo (1983a).

Habitat

Our microgeographical study of wild emmer was conducted at the Tabigha site near the sea of Galilee at Mediterranean sea level. The mean annual rainfall at the site is 436 mm; (during the pre-experimental year 1982/3, it was 583.1 mm; in 1983/4, 408 mm; in 1984/5, 458 mm). Table 1 presents the monthly distribution of rainfall at the site. The 1983/4 season was clearly drier, with rain starting in November rather than in October. Consequently both the annual mean and the monthly distribution of rainfall show higher aridity and differential patterns. The annual mean temperature is 24.1 °C (mean ranges 15 °C and 32 °C in January and August, respectively).

Table 1. Rainfall distribution (mm) at Tabigha during the two experimental periods. Measurements were obtained from the nearest meteorological station

Month	1983/84	1984/85
September	0	0
October	1.0	15.5
November	78.0	68.6
December	29.7	76.0
January	94.1	62.5
February	36.2	172.0
March	99.1	11.0
April	70.0	52.0
May	0	0
Total	408.1	457.6

The test area comprises patches of Middle Eocene hard limestone weathering into terra rossa red soil and Upper Pleistocene basalt flows weathering into basalt soil (see Atlas of Israel 1970 for the geological map and structure and for the edaphic differentiation; see also Ravikovitch (1969) and Dan and Raz (1970) for descriptions of soil types and their map in Israel, and Rabinovitch-Vin and Orshan (1974a, b) for plant-soil relationships in the terra rossa soil). Plant formation varies according to the two soil types. On basalt, it comprises *Psoralea hirsuta* and *Echinops blancheanus* (climax of *Pistacia atlantica, Zizypus spina-Christi* and *Z. Lotus)*, with *Scolymus maculatus, Cichorium pumilum, Carthamus glaucus, Echium judaicum, Hirschfeldia incana, Sinapis arvensis* and the wild cereals *Brachypodium distachyum, Phalaris bulbosum, Avena sterilis, Hordeum bulbosom, H. spontaneum*, and abundant *Triticum dicoccoides* (primarily the black spike-morph). On terra rossa, it consists of *Andropogon hirsuta* and *Echinops viscosus* (climax *Zizypus spina-Christi* and *Z. Lotus)*, *Carlina corymbosa, Notobasis syriaca, Capparis spinosa, Erucaria myagroides* with *Ferula communis, Gundelia Tournefortii, Phagnalon rupestre, Varthemia iphionoides, Dianthus multipunctatus*, and the wild cereals *Hordeum bulbosum, H. spontaneum*, and *Triticum dicoccoides* (less abundant than on basalt, and primarily the yellow spike morph).

On terra rossa, the vegetation present was sparser, lower in height, and earlier in both ripening and drying-up than that found on basalt. The soil layer was also shallower, and numerous bare rocks predominated the terrain interdispersed with pockets of soil varying in depth, especially in transect I. The terra rossa soil niche appeared, therefore, to be clearly drier and narrower than that of the basalt in terms of availability of water and moisture throughout the growing season (November to May). Superimposed on these soil characteristics, the topography of the terra rossa is more hilly than that of the flat, deeper soil, and southwestern dipping basalt. This contrast is by far more pronounced in transect I than in transect II.

Sampling and electrophoresis

We electrophoretically assayed 356 individual plants of wild emmer for genetic variation at 47 loci in all transects. One pair of transects was sampled in May 1984; the other in May 1985. Transects of each pair were 200 m apart; transect II, 50 m higher than transect I, and each transect was 100 m long. Each transect was equally divided into 50 m of red terra rossa and 50 m of brown basalt across a sharp geological boundary. Seeds were collected from 50 plants, about 1 m apart from each other, in each half of each transect. Sequential numbers 1–50 were for terra rossa plants and 51–100 for basalt plants. This sequence was used for mapping and correlation analysis. Leaf and root tissues of laboratory-germinated seedlings were homogenized and studied by starch gel electrophoresis following the sample preparation and enzyme assay methods described in Brown et al. (1978). The locus designations have been previously described (Nevo et al. 1982a).

Statistical analysis

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

Results

The allele frequencies and genetic diversities, *He*, at 16 common polymorphic loci of the 8 subpopulations, divided according to year, transect, and soil are presented in Table 2. The raw data is followed in the same table

by statistical analysis. The following main results are indicated.

Distribution of polymorphism among loci

Of the 47 loci, 23 proved to be totally monomorphic (*Aat-2*B, *3*A, *3*B; *Adh-1*B, *2*A, *2*B; *Cat*-A, B; *Est-4*A, *5*A, *Gdh*-A, B; *Mdh-1*B, *2; Nadh-1*A, *1*B, *2; Pepc; Pept-2; Pgi*-B, *Pgm*-B; *6Pgd-1*A, *1*B); 6 nearly monomorphic (*Aat-1*A, *1*B; *Est-4*B; *Ipor*-A, B; *Pept-1*A) and two polymorphic (*Gluc*-A, B) whose variants were distributed in a single patch, hence excluded from Table 2. The remaining 16 polymorphic loci (34%) appear in Table 2.

Distribution of alleles

Several loci varied significantly in allele frequency between soil types, transects, and years (9 out of 16, significant Chi-square tests for soils in Table 2). Figure 1 a–f illustrates the spatial distribution of several polymorphic loci that differ between the two soil types: *Pgm*-A, *Pgi*-A, *Ipol, Hk, Pept-3,* and *Est-5*B. These loci all displayed a nonrandom soil distribution of genotypes across transects and years.

Association with soil type

Several allozymes displayed consistent association (i.e., higher frequency) with soil type (Table 2, the ± in column 2). Six alleles showed associations with basalt, and three alleles with terra rossa. Eight of these nine associations were statistically significant as indicated earlier by χ^2 test (last column Table 2). Spearman rank correlations (r_s) were calculated between the presence of an allele and a dummy variable where basalt = 1, and terra rossa = 0. Likewise, estimates of r_s were also calculated with the position of the plant along the transect (first and third columns of statistical analyses in Table 2). Mean position (±S.E.) of each allele across the four transects appear between the two r_s in Table 2. The analysis with the position is sensitive not only to allele frequency for the soil types, but also to the distance from the contact zone between the two soil types.

All previous analyses have shown five alleles (*Ipo1*^{c}, *Aat-2*A^a, *Est-5*B^e, *Pgi*-A^a, and *Pgm*-A^{null}) to be associated (i.e., more frequently) with basalt and two alleles (*Est-5*B^a and *Hk*^{c}) with terra rossa. *Pept-3*^{b} also showed a consistent and significant association with terra rossa, but the analysis with the position resulted in contradictory results (for the reason, see Fig. 1 e). Therefore, the association of this allele with terra rossa needs confirmation.

Genetic summary

The genetic summary of the eight subpopulations appears in Table 3. The estimates of gene diversity, *He* (Nei 1975), based on all 47 loci were, on average, higher in the basalt than in the terra rossa (0.091, across transects and years, versus 0.075, respectively).

Discriminant analysis

We conducted stepwise discriminant analysis (SPSS-x 1986) maximizing the overall multivariate *F* ratio of the two soil microniches, based on a multilocus analysis involving seven polymorphic loci and nine alleles (*Pgm*-A^{null}, *Pgi*-A^a, ^{b}; *Mdh-1*A^b; *Pept-1*B^b; *Aat-2*A^a; *Acph-3*^{a}, ^{c}; *6Pgd-2*^{a}). These 7 loci were selected out of the 16 polymorphic loci tested in order to minimize the reduction in sample size for this analysis due to missing data. The results based on 7 chosen alleles (Table 4 B) are given in Fig. 2 and Table 4.

The analysis succeeded in significantly differentiating plants from the two soil types by a pairwise comparison ($F = 7.894$, *d.f.* 7/218, $P < 0.0001$, Table 4 A) and by a canonical discriminant function (Table 4 C and Fig. 2). Essentially the same results were obtained by analyzing separately each year, based on four – five alleles, with the same three first chosen alleles. The correct classification of plants into their soil microniche was 73% for the overall analysis (167 out of 229 plants analyzed; 63% on basalt, and 84% on terra rossa, Table 4 D). Similarly, the overall correct classification was 79% and 73% for 1983/4 and 1984/5, respectively. These figures are greater than those expected by chance, on the assumption of a random sample of neutral alleles.

A second discriminant analysis conducted on the allele frequencies in the eight subpopulations succeeded in differentiating significantly between the two soil types, using three (*Pgm*-A^{null}, *6Pgd-2*^{a} and *Pgi*-A^b) out of 11 alleles that entered into the analysis ($F = 134.27$, *d.f.* 3/4, $P = 0.0002$).

Analysis of genetic diversity, He, within and between populations

Gene diversity (Ht) of a subdivided population can be analyzed into its components, i.e., 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, mean gene diversity in the total population; and Ht = Hs + Dst (Nei 1973). Dm can be used for comparing the degrees of gene differentiation in different organisms. Considering each of the four "half transects" as a subpopulation (Table 2), the Dst analysis of gene diversity of the 12 polymorphic loci that were scored in both transects and both years is given in Table 5. On average, 73.9% of the total genetic diversity in the eight subpopulations exists within, and 26.1% between, subpopulations. These results indicate a

Table 2. Allele frequencies at 16 polymorphic loci of 8 subpopulations of *Triticum dicoccoides* (two soil types in two transects in 2 years) in Tabigha, Israel. For meaning of symbols, see legends below table

Year			1983/4				1984/5					Statistical analysis					
Transect			I		II		I		II			Spearman rank Correlation with basalt[a]	Average position			Spearman rank Correlation with position	χ^2 between soil types (1 *d.f.*)
Soil type			TR	B	TR	B	TR	B	TR	M	Mean		$\bar{X}$	S.E.	*N*		
Sample size (*N*)			47	42	31	50	49	44	47	46	356						
Locus allele + −		Compl. allele	Allele frequencies														
*Aat-2*A	a+++	(b)	0.087	0.205	0.0	0.120	0.0	0.0	0.149	0.239	0.103	0.129*	64	3.782	35	0.146**	4.989*
He			0.159	0.326	0.0	0.211	0.0	0.0	0.254	0.364	0.164						
N			46	39	31	50	49	41	47	46	349						
Acph-x	b−	(a)	0.568	0.111[b]	–	1.000	–	0.464	0.222	–	0.483	0.242**	54	3.087	69	0.279***	7.450**
He			0.491	0.198	–	0.0	–	0.497	0.346	–	0.306						
N			37	9		24		28	45		143						
Acph-3	a−	(b)	0.500	0.278	0.0	0.0	0.0	0.0	0.0	0.0	0.085	−0.112[c]	40	4.039	24	−0.111[c]	3.162[c]
	c+++−		0.0	0.185	0.032	0.036	0.526	0.286	0.0	0.022	0.136	−0.009 ns	50	4.412	40	−0.012 ns	0.0 ns
He			0.500	0.600	0.062	0.069	0.499	0.408	0.0	0.043	0.273						
N			35	27	31	28	38	42	47	46	294						
*Adh-1*A	a++	(b)	0.842	0.870	–	0.286[b]	0.051	0.571	0.0	0.0	0.262	0.133[c]	56	3.724	48	0.081 ns	2.653 ns
He			0.266	0.227	–	0.408	0.097	0.490	0.0	0.0	0.213						
N			19	23		21	39	7	31	46	183						
Est-1	b+−		0.250	0.818	1.000	0.938	–	–	–	–	0.896	0.050 ns	59	3.391	60	0.101 ns	0.0 ns
	c−		0.750	0.182	0.0	0.0	–	–	–	–	0.075	−0.143 ns	41	14.006	5	−0.165 ns	0.473 ns
(e=null)	e+		0.0	0.0	0.0	0.063	–	–	–	–	0.030	0.131 ns	71	11.000	2	0.073 ns	0.105 ns
He			0.375	0.298	0.0	0.117	–	–	–	–	0.197						
N			4	11	20	32					67						
*Est-2*A	a+	(b)	0.220	0.380	–	0.294[b]	–	–	–	–	0.283	0.146 ns	49	6.732	23	−0.002 ns	0.967 ns
He			0.343	0.471	–	0.415	–	–	–	–	0.410						
N			41	25		17					83						
*Est-2*B	a−		0.183	0.080	–	0.063[b]	–	–	–	–	0.128	−0.176 ns	37	11.780	10	−0.150 ns	1.113 ns
(a=null)	b+		0.0	0.120	–	0.0[b]	–	–	–	–	0.037	0.195[c]	78	11.724	3	0.203[c]	1.384 ns
	c−		0.549	0.520	–	0.500[b]	–	–	–	–	0.530	−0.036 ns	54	4.022	43	0.164 ns	0.014 ns
	d+		0.268	0.280	–	0.438[b]	–	–	–	–	0.305	0.080 ns	43	5.055	25	−0.153 ns	0.230 ns
He			0.593	0.630	–	0.555	–	–	–	–	0.593						
N			41	25		16					82						
*Est-5*B	a−−−		0.202	0.195	0.700	0.190	–	0.0[b]	0.936	0.413	0.372	−0.338***	41	2.440	107	−0.330***	26.760***
(a=null)	c−++		0.043	0.0	0.0	0.190	–	0.049[b]	0.0	0.283	0.085	0.241***	80	4.387	22	0.282***	16.856***
	e+++		0.755	0.805	0.300	0.619	–	0.951[b]	0.064	0.304	0.543	0.184**	57	2.513	118	0.153*	8.011**
He			0.387	0.314	0.420	0.544	–	0.093	0.120	0.657	0.362						
N			47	41	30	42		41	47	46	294						

Hk	a+−		0.077	0.692	1.000	0.0	0.0	0.0	0.0	0.0	0.081	0.046 ns	58	7.628	17	0.100 ns	0.169 ns
	b−++		0.250	0.0	0.0	1.000	0.278	1.000	1.000	1.000	0.693	0.340***	53	2.327	145	0.293***	22.751***
	c−−		0.673	0.308	0.0	0.0	0.722	0.0	0.0	0.0	0.226	−0.396***	27	2.889	47	−0.383***	31.088***
He			0.479	0.426	0.0	0.0	0.401	0.0	0.0	0.0	0.163						
N			26	13	6	34	36	2	47	46	210						
Ipol	c+++	(b)	0.0	0.158	0.091	0.387	0.0	0.0	0.0	0.326	0.121	0.309***	75	3.735	32	0.304***	23.464***
He			0.0	0.266	0.165	0.475	0.0	0.0	0.0	0.440	0.168						
N			9	19	22	31	49	42	47	46	265						
*Mdh-1*A	b++−−	(a)	0.0	0.189	0.233	0.800	0.277	0.0	0.021	0.0	0.202	0.175***	58	3.346	68	0.116*	9.405**
He			0.0	0.307	0.358	0.320	0.400	0.0	0.042	0.0	0.178						
N			38	37	30	50	47	41	47	46	336						
*Pept-1*B	b+++−	(a)	0.170	0.500	0.0	0.261	0.238	0.459	0.489	0.261	0.324	0.071 ns	60	2.606	94	0.178**	1.272 ns
He			0.283	0.500	0.0	0.386	0.363	0.497	0.500	0.386	0.364						
N			44	33	6	46	42	37	45	46	299						
Pept-3	b−−−−	(c)	0.311	0.286	0.737	0.408	0.429	0.185	0.955	0.512	0.448	−0.181**	54	2.424	111	−0.073ns	7.263**
He			0.428	0.408	0.388	0.483	0.490	0.302	0.087	0.500	0.386						
N			37	28	19	49	28	27	22	41	251						
Pgi-A	a++++	(b)	0.136	0.463	0.065	0.120	0.146	0.571	0.277	0.409	0.274	0.243***	65	2.251	94	0.286***	19.632***
	c+		0.0	0.049	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.075 ns	79	18.500	2	0.072 ns	
He			0.236	0.545	0.121	0.211	0.249	0.490	0.400	0.483	0.342						
N			44	41	31	50	48	42	47	44	347						
Pgm-A null	++	(a)	0.0	0.0	0.0	0.340	0.0	0.0	0.0	0.174	0.072	0.281***	87	1.699	24	0.353***	23.400***
He			0.0	0.0	0.0	0.449	0.0	0.0	0.0	0.287	0.092						
N			45	38	31	50	49	42	47	46	348						
6Pgd-2	a−−−+	(c)	0.261	0.225	0.267	0.220	0.190	0.125	0.021	0.043	0.164	−0.036 ns	48	3.380	55	−0.071 ns	0.097 ns
He			0.386	0.349	0.391	0.343	0.308	0.219	0.042	0.083	0.265						
N			46	40	30	50	42	40	47	46	341						

Monomorphic loci: *Aat-2*B, *3*A, *3*B, *Adh-1*B, *2*A, *2*B, *Cat*-A, B, *Est-4*A, *Est-5*A, *Gdh*-A, B, *Mdh-1*B, *2*, *Nadh-1*A, *1*B, *2*, *Pepc*, *Pept-2*, *Pgi*-B, *Pgm*-B, *6Pgd-1*A, *1*B
Low polymorphic loci: *Aat-1*A, *1*B, *Est-4*B, *Gluc*-A, B, *Ipor*-A, B, *Pept-1*A
Missing data. In most cases sample sizes range from $N = 19-50$. However, due to technical reasons some enzymes had low N. This does not include the monomorphic loci, except *Pept-2*; whereas *Est-4*A, B did not show upon gels of transect I-1984.
Abbreviations: TR = terra rossa; B = basalt; Compl. = complementary; (+−) = comparison of the frequency on basalt with that on terra rossa in the same year and transect: + = higher frequency on basalt; − = higher frequency on terra rossa; − = missing data of frequency
[a] = Basalt was designated as 1 and terra rossa as 0 for the correlation analysis. Degrees of freedom are N of that locus, minus 2
[b] = Excluded from analyses based on subpopulation frequencies, to balance missing data
Significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; [c] = $P < 0.10$; ns = $P > 0.10$

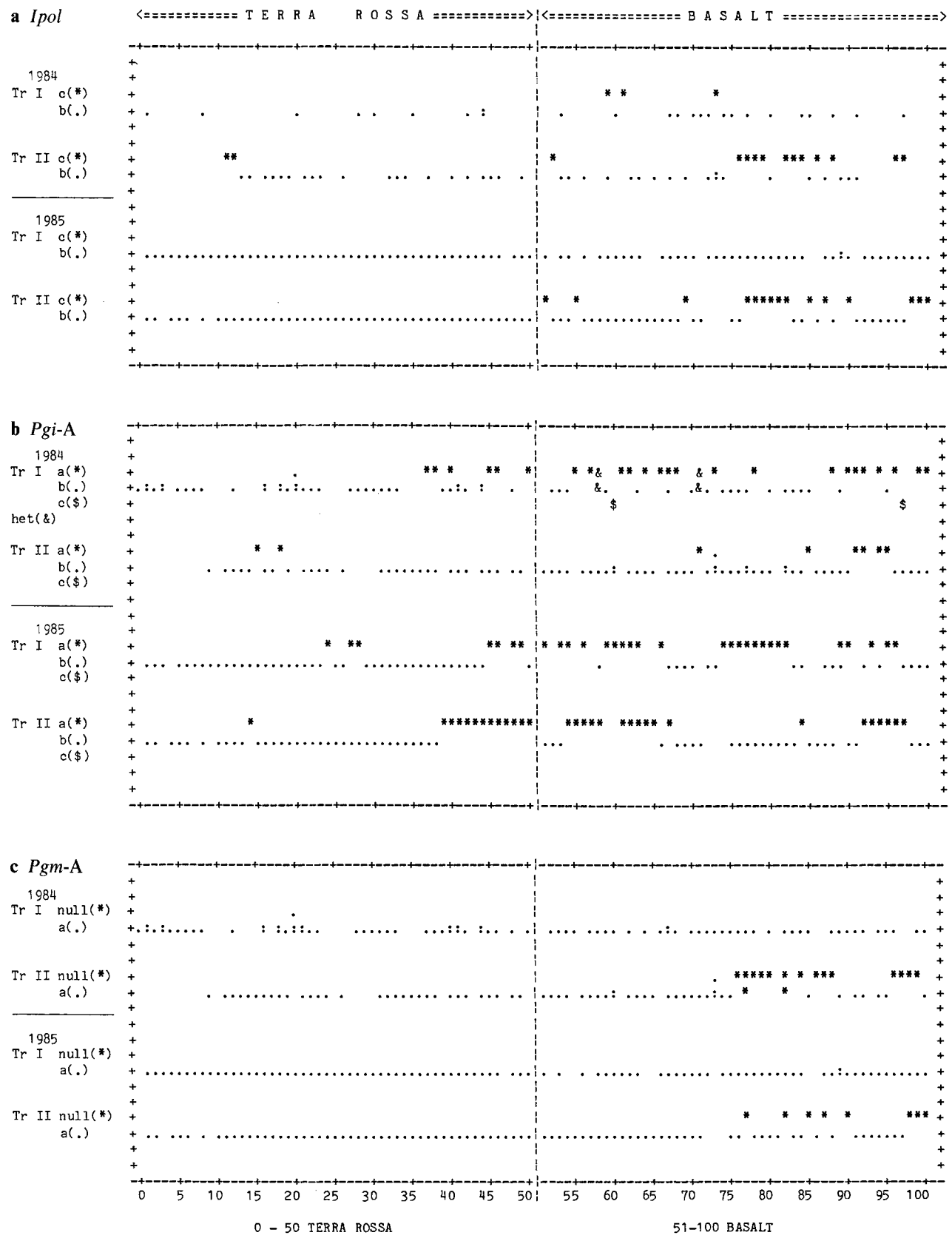
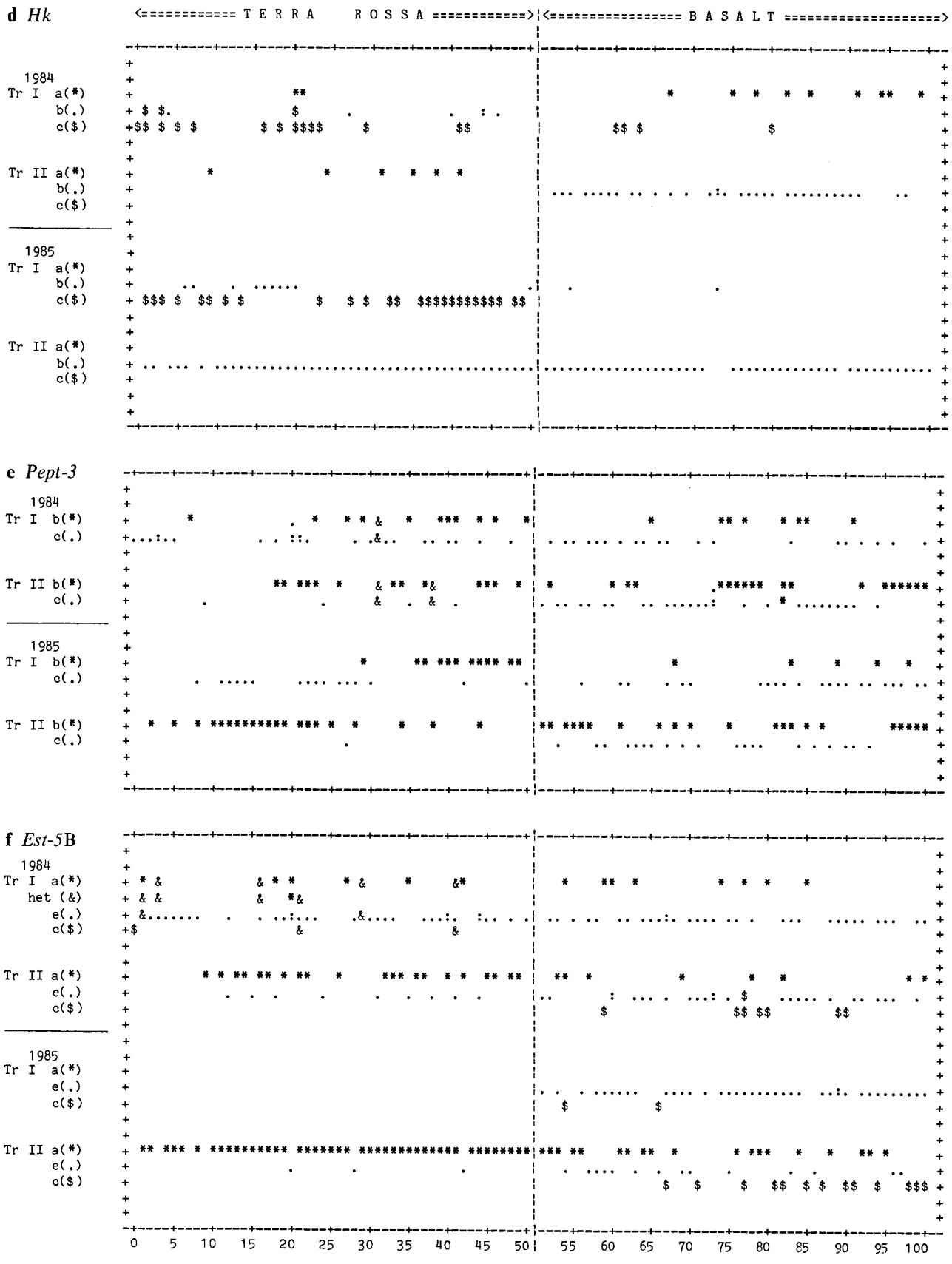


Fig. 1 a–f. Allele distribution of selected allozyme loci along the 100 m, two-soil-type transects at Tabigha, Israel. **a** *Ipol*; **b** *Pgi*-A; **c** *Pgm*-A; **d** *Hk*; **e** *Pept-3* **f** *Est-5*B


d Hk
<========== T E R R A R O S S A ==========>
<================= B A S A L T ==================>
1984
Tr I a(*)
b(.)
c($)
Tr II a(*)
b(.)
c($)
1985
Tr I a(*)
b(.)
c($)
Tr II a(*)
b(.)
c($)
e Pept-3
1984
Tr I b(*)
c(.)
Tr II b(*)
c(.)
1985
Tr I b(*)
c(.)
Tr II b(*)
c(.)
f Est-5B
1984
Tr I a(*)
het (&)
e(.)
c($)
Tr II a(*)
e(.)
c($)
1985
Tr I a(*)
e(.)
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Tr II a(*)
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c($)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
0 - 50 TERRA ROSSA
51-100 BASALT

strong genetic differentiation between subpopulations which is higher than the degree of relative differentiation found in the microgeographic analysis of allozymes (Nevo et al. 1981) and hordein (Nevo et al. 1983) conducted in wild barley at the same site several years earlier. The contrasting soils contributed 18% to the differentiation, but in certain loci (*Ipol, Pgi*-A, *Pgm*-A), the soil contribution was considerably greater. The ranking of the contribution of the three main factors to genetic differentiation between subpopulations was transect 27%, soil 18% and year 16%. The unaccounted percentages are due to various levels of interaction. Thus, allozyme differentiation in wild emmer wheat between soil types and other factors in substantive, is was also displayed in the previous discriminant analysis.

Genetic differentiation at two locus level

Gametic phase disequilibra (D) and relative D (D′) for the entire sample and for each soil type separately are given in Table 6. Ds can be classified into general (occurring in both soil types) and specific (occurring either on basalt or on terra rossa). Examples of general Ds were: *Adh-1*A^a – *Acph-3*^{e}; *Hk*^{a} – *Est-5* B^e. Specific Ds for basalt were: *Ipo1*^{b} – *Pgm*-A^a (D = 0.104***); *Mdh-1*^{a} – *Acph-x*^{a} (D = 0.152***); *Pept-3*^{c} – *Pept-1*B^b (D = 0.075***); *Hk*^{c} – *Acph-3*^{a} (D = 0.039***). Specific Ds for terra rossa were: *Hk*^{b} – *Pept-1*B^b (D = 0.107***); *Mdh-1A*^{a} – *Gluc*-B^a (D = 0.064***); *Pept-3*^{b} – *Est-5*B^a (D = 0.130***); Pgi-A^b – Acph-x^a (D = 0.088***). (Significance levels: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; calculated by

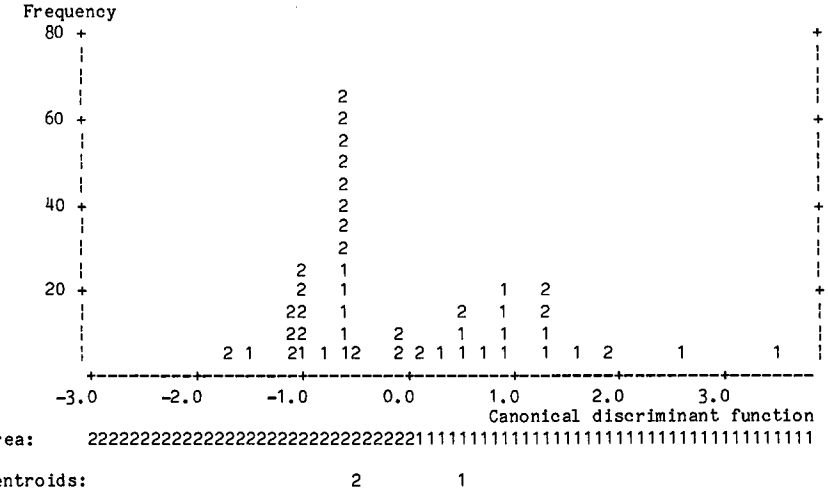

Fig. 2. Discriminant analysis of wild emmer wheat in Tabigha, according to soil type, based on seven polymorphic allozyme loci and nine alleles. 1 = Basalt; 2 Terra rossa

Table 3. Summary of genetic variation based on 47 loci of *Triticum dicoccoides* in 8 subpopulations according to soil, transect and year, at Tabigha

Subpopulation			Sample size (N)	Mean no. of alleles per locus (A)	Mean proportion of loci				Genetic diversity (He)
	Transect	Soil type			Polymorphic per population (P)		Heterozygous per individual (H)		
					(1%)	(5%)	Mean	S.E.	
1983/4									
1.	I	Basalt	42	1.457	0.370	0.370	0.005	0.002	0.130
2.	I	Terra rossa	47	1.447	0.362	0.298	0.008	0.003	0.112
3.	II	Basalt	50	1.279	0.256	0.233	0.001	0.001	0.084
4.	II	Terra rossa	31	1.171	0.171	0.146	0.003	0.003	0.046
1984/5									
5.	I	Basalt	44	1.175	0.175	0.175	0.0	0.0	0.073
6.	I	Terra rossa	49	1.308	0.308	0.308	0.0	0.0	0.100
7.	II	Basalt	46	1.233	0.209	0.163	0.0	0.0	0.075
8.	II	Terra rossa	47	1.182	0.182	0.114	0.0	0.0	0.041
Total			356	1.281	0.254	0.226	0.002	0.001	0.083

Table 4. Stepwise discriminant analysis of genetic diversity of *Triticum dicoccoides* between two soil types of Tabigha

A. Pairwise comparison between basalt and terra rossa. The F statistic has 7 and 218 degrees of freedom

	Basalt
Terra rossa	$F = 7.89$ $P < 0.00005$

$N = 226$ (out of 356); 118 on basalt, 108 on terra rossa

B. Summary table (chosen variables)

Action			Variable order	Wilks' Lambda	Significance
Step	entered	allele			
1	*Pgm*-A	null	1	0.92784	< 0.00005
2	*Pgi*-A	a	2	0.85851	< 0.00005
3	*Mdh-1*A	b	3	0.82996	< 0.00005
4	*Acph-3*	a	4	0.81591	< 0.00005
5	*Aat-2*A	a	5	0.80718	< 0.00005
6	*Acph-3*	c	6	0.80282	< 0.00005
7	*Pept-1*B	b	7	0.79779	< 0.00005

C. Canonical discriminant function

Function	Eigenvalue	Canonical correlation	After function	Wilks' lambda	Chi-squared	*d.f.*	Significance
1	0.25347	0.4497	0	0.7978	49.814	7	< 0.00005

D. Classification results

Actual group	No. of cases	Predicted group membership	
		1	2
1. Basalt	118	74 62.7%	44 37.3%
2. Terra rossa	111	18 16.2%	93 83.8%

72.93% of plants correctly classified

Table 5. Partition of genetic diversity of *Triticum dicoccoides* between soil types, years and transects at Tabigha, based on 12 polymorphic loci (Gst analysis: Nei 1973)

Locus	Alleles	Sample	Ht	Hs	Dst							Gst	Dm	Rst
					Total	(%)	Transect	(%)	Years	(%)	Soil (%)			
*Aat-2*A	2	349	0.1850	0.1697	0.0153	100	0.0024	16	0.0001	1	0.0031 20	0.0826	0.0175	0.1030
Acph-3	3	294	0.3676	0.2572	0.1104	100	0.0656	59	0.0159	14	0.0022 2	0.3003	0.1261	0.4904
Gluc-A	2	295	0.0904	0.0640	0.0264	100	0.0042	16	0.0030	11	0.0042 16	0.2920	0.0302	0.4713
Gluc-B	2	295	0.0965	0.0706	0.0259	100	0.0049	19	0.0024	9	0.0048 19	0.2683	0.0296	0.4191
Hk	3	210	0.4622	0.1544	0.3078	100	0.2092	68	0.0317	10	0.0521 17	0.6660	0.3518	2.2784
Ipol	2	265	0.2123	0.1646	0.0478	100	0.0149	31	0.0070	15	0.0203 42	0.2249	0.0546	0.3316
*Mdh-1*A	2	336	0.3228	0.1752	0.1477	100	0.0120	8	0.0365	25	0.0099 7	0.4575	0.1688	0.9637
*Pept-1*B	2	299	0.4383	0.4031	0.0352	100	0.0000	0	0.0031	9	0.0022 6	0.0804	0.0403	0.0999
Pept-3	2	251	0.4946	0.4087	0.0859	100	0.0380	44	0.0048	6	0.0157 18	0.1738	0.0982	0.2403
Pgi-A	3	347	0.4059	0.3447	0.0612	100	0.0049	8	0.0095	16	0.0241 39	0.1508	0.0700	0.2030
Pgm-A	2	348	0.1334	0.1025	0.0309	100	0.0103	33	0.0018	6	0.0101 33	0.2317	0.0353	0.3446
6pgdh-2	2	341	0.2745	0.2583	0.0162	100	0.0028	17	0.0112	69	0.0002 2	0.0592	0.0186	0.0719
Mean	2.3	307	0.2903	0.2144	0.0759	100	0.0308	27	0.0106	16	0.0124 18	0.2615	0.0868	0.4049

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

Chi-square with Yates correction and excluding all heterozygotes). D' displayed a pattern similar to D except at very low values of D. Notably, in some cases, such as *Pgi*-A^b – *Acph-x*^{a}, the sign of D was opposite in the two soil types, a pattern not revealed by D'.

Multilocus organization – terra rossa versus basalt

The 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 and combines all paired-loci gametic phase disequilibria. We calculated multilocus organization indices for the entire data set of wild emmer at Tabigha, as well as for each transect separately, and our results are presented in Table 7. Notably, samples of the two years represent the same local seed

Table 6. Representative gametic phase disequilibria, (D) and (D'), between paired loci of *Triticum dicoccoides* in Tabigha: a, consistant; b, mainly on basalt; c, on terra rossa; reported on the entire sample, and separately for each soil type

Locus & allele	Locus & allele	Over all		Basalt		Terra rossa	
		D	(D') p	D	(D') p	D	(D') p
a. Consistant disequilibria							
*Adh-1*A a	*Acph-3* a	0.082	(0.83)***	0.066	(0.91)***	0.099	(0.80)***
*Adh-1*A a	*Est-5*B e	0.111	(0.65)***	0.079	(0.54)**	0.171	(0.81)***
Hk b	*Acph-3* b	0.092	(0.81)***	0.083	(0.88)***	0.087	(0.75)***
Hk a	*Est-5*B e	0.048	(0.76)***	0.048	(0.81)**	0.049	(0.72)**
6Pgd-2 a	*Adh-1*A a	0.044	(0.37)**	0.038	(0.37)[a]	0.053	(0.38)*
*Adh-1*A a	*Pept-3* c	0.076	(0.57)***	0.086	(0.77)**	0.062	(0.38)[a]
Pept-3 b	*Est-2*A b	0.055	(1.0; c)*	0.054	(1.0; c) n.s.	0.036	(1.0; c) n.s.
Pept-3 b	*Est-2*B c	0.061	(0.62)*	0.043	(0.59) n.s	0.069	(0.61) n.s.
b. Mainly on basalt							
*Adh-1*A a	*Pept-1*B b	0.023	(0.14) n.s.	0.060	(0.34)*	−0.016	(0.20) n.s.
Pept-3 c	*Pept-1*B b	0.056	(0.50)***	0.075	(0.69)***	0.020	(0.18) n.s.
Hk c	*Acph-3* a	0.034	(0.68)***	0.039	(1.0; c)***	0.023	(0.44) n.s.
Ipol b	*Pgm*-A a	0.062	(0.86)***	0.104	(0.85)***	–	
Ipol b	*Pgi*-A a	0.022	(0.66)*	0.060	(0.73)**	0.003	(1.0; c) n.s.
Ipol c	*Est-5*B c	0.042	(0.47)***	0.055	(0.41)**	–	
Ipol c	*Pept-3* b	0.017	(0.21) n.s.	0.058	(0.36)**	–	
*Mdh-1*A a	*Acph*-x a	0.087	(0.92)***	0.152	(1.0; c)***	−0.005	(1.0; c) n.s.
*Pept-1*B b	*Est-2*A b	0.054	(0.69)*	0.079	(0.60)*	0.040	(1.0; c) n.s.
Pgi-A b	*Mdh-1*A b	0.031	(0.53)**	0.061	(0.57)***	0.016	(0.78) n.s.
c. Mainly on terra rossa							
Pgi-A b	*Acph*-x a	0.039	(0.24)[a]	−0.052	(0.24) n.s.	0.088	(0.64)***
*Adh-1*A a	*Acph*-x a	0.037	(0.16) n.s.	−0.099	(0.69) n.s.	0.095	(0.52)*
Pept-3 b	*Est-5* B a	0.070	(0.40)***	0.014	(0.10) n.s.	0.130	(0.65)***
Hk c	*Est-5*B e	0.023	(0.29) n.s.	−0.008	(0.41) n.s.	0.066	(0.45)*
Hk b	*Pept-1*B b	0.054	(0.64)***	−0.005	(0.07) n.s.	0.107	(0.79)***
Hk c	*Pept-1*B a	0.036	(0.59)*	−0.016	(1.0; c) n.s.	0.084	(0.75)***
Hk b	*Pept-3* b	0.027	(0.17) n.s.	−0.004	(0.07) n.s.	0.092	(0.50)**
*Mdh-1*A a	*Gluc*-B a	0.031	(0.75)***	–		0.064	(0.76)***
Acph-3 a	*Est-5*B e	0.024	(0.46)***	0.003	(0.14) n.s.	0.064	(0.61)***
Acph-3 b	*Est-5*B a	0.046	(0.60)***	0.020	(0.44) n.s.	0.071	(0.67)***
6Pgd-2 a	*Est-2*A a	0.047	(0.24)[a]	0.028	(0.13) n.s.	0.063	(0.39)[a]
Acph-x a	*Est-5*B a	0.050	(0.28)*	−0.009	(0.21) n.s.	0.051	(0.23)[a]

Significance: * $= P < 0.05$; ** $P < 0.01$; *** $= P < 0.001$; [a] $= P < 0.10$; n.s. $= P > 0.10$; 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)

pools. The observed variance in the entire data set was 221% above the expected. Multilocus organization appears to be higher in emmer growing on terra rossa than on basalt. The standardized intensity index of multilocus structure X(2) was 1.16 for wild emmer on terra rossa, and 0.84 for that on basalt. In other words, the observed variance in K was on terra rossa 116%, significantly higher than expected, and on basalt only 84%, nonsignificantly higher than expected. The difference between the observed and expected variance of s^2K was significant in transect 2 in both years (1983/4–1984/5), but was nonsignificantly opposite in Transect 1 (Table 7). We conclude that, on average, multilocus organization intensity is higher in wild emmer growing on terra rossa than in that growing on basalt.

Discussion

Genetic differentiation in wild emmer

Genetic differentiation of wild emmer wheat was first observed in regional studies (Nevo et al. 1982a) demonstrating geographical differentiation and ecological associations of allozymic diversity on a regional scale. We then demonstrated that allozyme patterns were significantly correlated with, and partly predictable by, water

Table 7. Estimates of multilocus genetic organization of *Triticum dicoccoides* in two soil types at Tabigha

Soil type	Polymorphic loci	Expected variance of K	Upper limit of 95%	Estimate of variance of K[a]	X(2)[b]
Entire sample					
	18	0.791	< 1.31	2.54*	2.21
Over all					
Terra rossa	17	3.193	6.28	6.91*	1.16
Basalt	16	3.490	7.17	6.41	0.84
Transect 1					
Terra rossa	15	3.228	7.63	6.17	0.91
Basalt	15	3.149	7.35	6.87	1.18
Transect 2					
Terra rossa	10	1.752	2.71	3.70*	1.11
Basalt	14	2.858	4.35	4.57*	0.60

Significance: * = $P < 0.05$

[a] K = number of heterozygous loci in two randomly chosen gametes

[b] X(2) = measure of multilocus structure

availability factors and soil types. Even at that early stage, we noticed that genetic differentiation occurs both at the regional and local levels, i.e., macro- and microscales. The genetic distance (Nei 1972) between the two geographically close populations of Qazrin and Yehudiyya, separated by 10 km, was $D = 0.248$, a value similar to the genetic distances between many sibling species. The striking multilocus differentiation at that site, involving two fundamentally different genotypes as defined by eight loci (*Mdh-1, Ipol, β-Glu, Pept-1, Pept-3, 6Pgdh-2, Hk,* and *Rc*) has been studied in depth (Golenberg 1986; Golenberg and Nevo 1987).

Golenberg (1986) found that gametic phase disequilibria between loci increases over four generations, displaying probably two cases of epistatic selection. Significant differences were found among populations of the Yehudiyya and Qazrin genotypes for morphological, germination, and phenological characters when grown under standardized greenhouse conditions. Reciprocal transplantation and replacement series competition experiments did not, however, reveal any local adaptations in the year studied. Genetic neighborhood area was estimated to be about 5 m in diameter, implying that mean gene flow distance per generation is about 1.25 m (Golenberg 1987). Golenberg (1986) concluded that there is no strong evidence that multilocus structures are the units of selection in *Triticum dicoccoides* in this area. His results suggest that sporadic selection pressures, high selfing rates, and limited gene flow are of major importance in the evolution of multilocus associations involved in genetic differentiation of wild emmer.

Ecological factors affecting genetic differentiation at Tabigha

The present study demonstrated that three factors are associated with alloyzme differentiation: (i) yearly differences; (ii) topographic differences; and (iii) soil differences. We will comment briefly on each of these factors which seem to share a major ecological factor, namely, the aridity index, in addition to other factors. The first factor, mean annual rainfall, varies drastically between years in Israel (Atlas of Israel 1970, Maps K, L, M on page IV/2). Year to year fluctuations in our test locality at Tabigha vary from the lowest annual rainfall isopleth of 200 mm (once in the period 1931/2 – 1960/61), and less than 400 mm (8 times in that period) to 900 mm. These estimates of 200 and less than 400 mm are close to the aridity limit of dry farming of the main cereals in Israel. The total annual rainfalls in 1982/3, 1983/4 and 1984/5 in the area were 581 mm, 408 mm and 458 mm, respectively. Moreover, the monthly rainfall distribution differs distinctly between the two sampling seasons. The growing season of 1983/4 was dry in October (germination time!) and received low rainfall in December, while 35% of the annual rainfall of 1984/5 was in February (Table 1). Thus, the two years varied considerably in their aridity pattern.

Notably, some allele frequencies differ drastically between the two test years. It should be recalled that in *T. dicoccoides,* as in many other annual plants, there is a large seed pool in the soil. The germination of a yearly seed set is spread over several or many years, depending on the species. Thus, in each year, a sample of the seed pool is germinating, and the ecological and climatic selection pressures of that particular year affects the allele frequencies of the adult sample collected at the end of this growing season. Seed pools retard evolutionary change and act as the genetic memory of the selective pressures of previous years (Levin 1978; Templeton and Levin 1979). Consequently, sometimes even drastic changes between years take place, as was exemplified by a few loci in our study. This explanation may be particularly relevant since the Tabigha site is located near the 400 mm isopleth, which is the rainfall lower border of wild emmer wheat in Israel and possibly also in the Near East Fertile Crescent.

The second factor, topographical differences between the two transects that share the same soil types, may be also related to water availability differences. Transect I is steeper and lower altitudinally by about 50 m than transect II. Water content fluctuates more in the hilly and steeper transect I, particularly in the terra rossa section, than in relatively flat transect II. No measurements were taken, however, to register the differences between the two transects, and this assessment is based on the differ-

ent topographies causing higher runoff and drainage in transect I.

The third factor, soil type, also appears to contribute to ecological heterogeneity. The Kaolinitic terra rossa, derived from the hard limestone rocks of the Middle Eocene Bar Kokhba formation, is constantly deficient in water supply and rich in phosphates (Rabinovitch-Vin and Orshan 1974a, b; Rabinovitch-Vin 1983). The percentage of available soil moisture (field capacity-wilting point) in this soil is nearly half (9%, calculated per soil volume) that of other soil types in the region, including basalt. Total available phosphorous content is by far higher than in other soil types. Percolation of water is high and the upper soil layers dry up rapidly. These physical and chemical differences drastically affect the structure and phenology of plant formations on the two soils.

The differences in plant formations on the two soil types have been reported in the Materials and methods. The absence of *Sarcopterium spinosum,* the major component of batha (small bush plant formation), on this kaolinitic terra rossa is probably due to an interaction between deficiencies of water supply and carbonates. Phenologically, the annual plants growing on terra rossa terminate their life cycle and dry up in the spring, at most, 3–4 weeks after the last effective rains. This is in contrast to the 8–10 weeks on other soil types, including basalt. In contrast to terra rossa, the brown basalt soil (Dan and Raz 1970; A. Rabinovitch-Vin, personal communication) is subject to extremes of high and low water content due to its montmorinolitic clays. Hence, its water regime is more broad-niched, i.e., subject to fluctuations in water content, than the narrow-niched kaolinitic terra rossa. These differences are clearly reflected in the different plant formations on the two soil types, which display the long-term biotic soil differentiation (see Material and methods).

Thus, the basic differentiating factors – yearly fluctuations, topography, drainage, and soil type – seem to share a basic component of higher aridity stress on terra rossa, in transect I, in 1983/4, than in their counterparts. However, the range of fluctuation in water availability is higher on basalt and in transect I than on terra rossa and in transect II where relatively constant narrow range of moisture prevail in large part of the growing season. Additional correlative factors, physical as well as biotic, are surely involved as differentiating agents. However, water availability, or the aridity index, appears to be one of the major, if not the major differentiating stress factor in the alternative soils, topographies, and years at Tabigha. Since Tabigha is situated near the limit of dry farming of the main cereals in Israel, fluctuations in water availability, or changes in the aridity stress, seem to predominate in that region as a factor causing local genetic differentiation.

Genetic differentiation at single and multilocus levels by natural selection in wild emmer at Tabigha

Our present results suggest that genetic diversity and differentiation at both the single and multilocus structures in Tabigha are subject to selection. This is indicated by: (i) single loci (Table 2) and multilocus soil differentiations (Fig. 2 and Table 4); (ii) higher *He* in basalt and transect I in accordance with the prediction of the niche-width variation hypothesis; (iii) soil-specific two locus gametic phase disequilibria (Table 6); and (iv) soil-specific multilocus genome organization (Table 7). The following are details of each point.

The differentiations in the levels of heterozygosity of individual loci and in overall genetic diversity, *He,* support the niche-width variation hypothesis (Van Valen 1965). *He* was higher in 1983/4, in transect I, and in the basalt soil, as expected by the niche-width variation hypothesis which predicts a positive correlation between genetic diversity and ecological heterogeneity. First, for 41 shared loci (see Table 2), *He* was higher in 1983/4 than in 1984/5, 0.073 versus 0.064, respectively. In drier years (like 1983/4), we assume a larger microspatial variance in the aridity stress at the different microsites. Second, *He* was higher in transect I than in transect II, 0.085 versus 0.052, respectively; and third, *He* was higher on basalt than on terra rossa, 0.076 versus 0.061, respectively. For basalt, higher *He* was also apparent in individual loci (e.g., *Ipol, Pgm*-A, *Pgi*-A, *Aat-2*A), but not in others, (e.g., *Pept-1*B; Table 2).

A criticism of our statistical analysis could be that the linkage disequilibrium and genome organization estimates reported here, are, in part, influenced by the pooling of samples having different gene frequencies, Nei and Li (1973) showed that the pooled estimate of D when taken over subpopulations is a function of the covariance in gene frequencies and may be large even when there is no disequilibrium within subpopulations. This criticism seems irrelevant in our study. First, the 2-year repetitions derive from the very same seed pool, and, in the absence of climatic selection, may be viewed as two independent samples of the same population. Second, in the absence of edaphic selection, the two soil types represent a continuum of the very same population. Third, in the absence of topographic selection, even the transects are, in fact, two portions of the same population. Thus, our statistical analysis comparing and contrasting edaphic subsets (Tables 6 and 7), as well as our overall analysis of the entire local sample (consisting of 8 subsamples) are legitimate. In the absence of selection, we expect no differences in genetic patterning, either at the single or multilocus level, between the subsampled spatial or temporal sections. By contrast, any significant genetic differences are taken as evidence of selection. Despite this theoretical justification, we did check D in each of the individual

eight subsamples and found consistent Ds in the subsamples where those loci were polymorphic. Thus, our Ds, as analyzed in Table 6 (providing the basis of Table 7, of genome organization), appear natural, and generally exist in the subsamples.

The clear single and multilocus qualitative and quantitative differentiation between terra rossa and basalt suggest the operation of edaphic selection. This conclusion is reinforced by the observation of different gametic phase disequilibria characterizing each soil type (Table 6). This edaphic differentiation of D is certainly unexpected by chance, without selection, along such short transects and dense population stands, particularly since some of the seeds are dispersed across the transect by both ants and mice. Despite the average slow seed dispersal (Golenberg 1987), its effect is cumulative over many generations. In particular, Ofer (1980) has shown in his thorough study of the ecology of ant populations of the genus *Messor* that an estimate of the effective foraging range is more than 40 m. Finally, at the multilocus level, there is substantive multilocus organization (Brown et al. 1980). An inbreeding population, such as wild emmer, with a high multilocus variance (s^2K) will produce more multiple heterozygous individuals and more multiple homozygous individuals than a population with a lower variance. Such an inbred population produces fewer clones having greater genetic differentiation between them. This is precisely what we found by comparing clonal diversity: namely, different homozygous genotypes of wild barley *Hordeum spontaneum* across the same soil transect (Nevo et al. 1981 and unpublished results).

In our present wild emmer analysis, the variance was, on average, 116% and 84% higher than what was expected for random association in terra rossa and basalt genotypes, respectively (Table 7). Thus, the terra rossa genotype showed overall 32% more multilocus organization than the basalt genotype. Furthermore, while the terra rossa index (1.16) was significant, that of basalt (0.84) was not. We interpret this difference, although it is not significant here, to be a reflection of a higher selection of the more xeric terra rossa genotype of wild emmer as compared to the basalt genotype. Notably, multilocus organization proved significantly higher in the sunny microclimatic niche than in the shady one at the nearby Yehudiyya site (Nevo et al. 1988). Thus, in both cases, higher multilocus organization appears to be positively correlated with either edaphic or climatic selection.

It is noteworthy that genetic diversity in our macrogeographic analysis of many species, including wild wheat (Nevo and Beiles 1988), is also associated with higher climatic unpredictability in steppic regions, as is the variation in *He* at the Tabigha microsite discussed above. This correlation of He in wild emmer wheat, with a broader moisture niche, corroborates the niche-width variation hypothesis, both macro- and microgeographically. These results in wild emmer are in line with macrogeographic studies in 21 animal and plants species in Israel, involving *Triticum dicoccoides* and *Hordeum spontaneum*. There, *He* increases southwards towards the climatically more unpredictable steppic regions separating the northern Mediterranean and southern desert climatic regimes (Nevo 1983a; Nevo and Beiles 1988). Thus, microgeographic genetic differentiation mirrors the macrogeographic one, presumably caused in both cases by the unpredictability of the aridity stress.

Natural selection caused by stress

The effects of various stress factors as agents unravelling the operation of natural selection have been demonstrated earlier in microsite differentiation of allozymes in barnacles through temperature stress (Nevo et al. 1977) and through aridity stress in landsnails (Nevo et al. 1982b); and in wild barley, *Hordeum spontaneum* (Nevo et al. 1981, 1983, 1986); as well as in controlled laboratory experiments of pollution stress (reviewed in Nevo 1986). Aridity stress has been previously implicated in allozyme differentiation in many unrelated species in both Israel (Nevo 1983a) as in the world (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 in Hedrick 1982). In our present study, we do not have direct evidence implicating ecological selection on specific alloyzme loci rather than on linked blocks of genes. However, our evidence, summarized above (Nevo and Beiles 1988), supports the hypothesis that genetic differentiation by natural selection at the Tabigha microsite is affected at least partly by aridity stress on various phases of the life cycle of seeds, seedlings, and ripening plants.

Neutral model expectations

Are the observed allele frequency patterns in the subpopulations at Tabigha explicable by a simple neutral model? The neutral model would predict, especially in an inbreeder such as wild emmer, a random distribution of mother plants, each one propagating itself in its vicinity and creating the well-known neighborhood effect. Genotypic patches are expected to develop in plant populations when gene dispersal is limited (e.g., Turner et al. 1982). A spurious correlation of gene frequency with soil type could easily be observed with a limited number of samples. Does this confounding effect lead to our present results? Our evidence rules out the possibility that the patches that were found are random. In an inbreeder, the pattern will involve the entire genotype and not just the individual, separate alleles. While a neutral model ex-

pects different alleles in different clusters, it does not expect any systematic differential patterning of genotype clusters in size, distribution, or both, between soils, transects, and years. It is noteworthy that only factors such as population size and isolation by distance, without selection, may generate differential clustering patterns of genotypes.

The neutral model expectations are not met at Tabigha, and, therefore, a neutral model is highly unlikely here. The negation of the neutral model is based on the following reasons.

(i) Population size. The population under study involves thousands of wild emmer plants and does not represent a Wrightian small population of $N=10-100$.

(ii) Population density pattern. The density of wild emmer wheat on basalt is higher than that on terra rossa, though plant continuity characterizes both soil types.

(iii) Vegetation. Both plant association and plant species kind and diversity differ on both soil types, as described earlier in the present study and elaborated in Rabinovitch-Vin and Orshan (1974a, b). Likewise, spike color polymorphism in wild emmer varies between the two soil types, the yellow morph predominating on terra rossa, while the black morph is common on basalt.

(iv) Genotype clustering. Remarkably, we found distinct differential genotype clustering on both soil types at the same locality both in an earlier study on wild barley, *Hordeum spontaneum* (Nevo and Beiles, unpublished) and also in the present data set on wild emmer (in preparation). In both species of wild cereals, the *size* of repetitive genotype clusters was distinctly higher on terra rossa than on basalt. This was true in all four subsamples of wild emmer.

(v) Temporal differentiation. The differences in allele and genotype frequency and their distributional patterns between the two test years is also unlikely under a neutral model. The latter should consider the 2 years as two independent samples derived from the same seed pool at the same locality. The temporal yearly differentiation, as well as the differences within years between soil and transects, are highly unlikely under a neutral model. By contrast, points (ii), (iv), and (v) are clearly explicable under a climatic-selection model. Furthermore, we strongly suspect, and plan to test in the future, that microniche differentiation within each soil type is associated with selected clusters of genotypes. In other words, the neighbor effect phonomenon may be here primarily a reflection of ecological selection.

Conclusions and prospects

Our data seems to be inconsistent with the hypothesis that allozymic diversity is neutral, and that its fate in nature is primarily determined by mutation input and random fixation, as suggested by the neutral theory of molecular evolution (Kimura 1983). The allozymic patterns discovered here are structured in accordance with the ecological heterogeneity of the test site; presumably, predominantly by the aridity index. The genetic differentiation discovered is unlikely to be the result of random forces. The population size at Tabigha involves thousands of plants. The largest stands of wild emmer in Israel occur in the neighboring areas of the Golan Heights and eastern Upper Galilee Mountains, and Tabigha is located within this region. Neither migration nor neighborhood effects in the framework of a neutral model can explain the pattern unravelled. Migration is indeed low (Golenberg 1986), but continuous over many generations, and neighborhood effects vary adaptively between both soil types. Thus, the genetic differentiation encountered here on a very low scale suggests that natural selection seems to be an important differentiating factor of wild emmer at Tabigha, as elsewhere.

The higher levels of genetic diversity, *He*, and polymorphism, *P*, in 1983/4, on basalt, and in transect I, may be regarded as an adaptive strategy for increasing fitness in a year, soil type, and/or topography subject to a larger spatiotemporal microclimatic heterogeneity, in accordance with the niche-width variation hypothesis (Van Valen 1965). This result suports regional (Nevo 1983a) and global (Nevo et al. 1984) patterns where allozymic diversity is, in general higher with increasing spatiotemporal ecological heterogeneity and unpredictability.

Our empirical results are consistent with previous microsite experimental studies (e.g., Hamrick and Allard 1972; Hamrick and Holden 1979). They also corroborate the theoretical expectation 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 are affected by diversifying balancing selection on allozyme polymorphisms. Transplant experiments could test this hypothesis by studying the fitness of allozyme genotypes from alternative microniches (Jain and Rai 1980) as well as subtantiate their biochemical specificities and physiological functions (Nevo 1983c). Likewise, allele frequencies of gene pools in the soil should be compared and contrasted with those of the adults over several years in order to assess the temporal operation of selection. Transplant experiments have been conducted by Golenberg (1986) on the Yehudiyya and nearby Qazrin alternative multilocus allozyme genotypes, but the did not reveal differential fitness, possibly due to the short testing period and technical disturbances.

Our results not only have theoretical applications, but, more importantly, practical ones. If allozymic diversity indeed varies dynamically with the environment, then not only its macro- but its microgeographical structure is tractable by ecological factors. Such clues may

help in maximizing the exploitation, conservation, and utilization of wild emmer wheat in breeding (Brown and Clegg 1983; Nevo 1983b).

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