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Microclimatic stress and adaptive DNA differentiation in wild emmer wheat, *Triticum dicoccoides*

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Abstract Genetic diversity was examined by RAPD-PCR analysis in 118 registered individuals of wild emmer wheat, *Triticum dicoccoides*, from a microsite at Yehudiyya, northeast of the Sea of Galilee, Israel. The test involved two climatic microniches in the open oak-park forest of *Quercus ithaburensis*: (1) sunny between trees and (2) shady under the trees' canopies. Comparisons were based on 97 loci amplified by 20 oligonucleotide primers. Significant genetic differentiations were found at single-, two- and multilocus structures between the neighbouring shady and sunny niches. These DNA polymorphisms appear to be associated with microclimatic stresses. The pronounced niche-effect on the significance of linkage disequilibrium and niche-specific linkage disequilibrium may suggest that natural selection directed the two-locus associations. The structure of the multilocus associations also mainly results from natural selection, and not by chance from population subdivision, or founder effects. These findings are largely parallel to the previous allozymic results at single-locus and multilocus levels. Both the DNA and the allozymic results suggest that microclimatic selection appears to play an important role in DNA differentiation as well as in protein polymophism.

Key words Genetic diversity · Natural selection · RAPD-PCR · Microclimate stress · Emmer wheat · *Triticum dicoccoides*

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Introduction

Wild emmer wheat, *Triticum dicoccoides*, is a tetraploid predominantly self-pollinated plant, and the wild progenitor of modern tetraploid and hexaploid cultivated wheat (Zohary 1970; Feldman 1976; Kimber and Feldman 1987). In the past decades, phenotypic and genotypic variation of agronomically important traits within and between populations of wild emmer wheat has displayed high genetic diversity (Nevo and Beiles 1989) and identify rich genetic resources usable in wheat improvement (Nevo 1983 b, 1988 a, 1995 a). The ecological-genetic interface has also been studied extensively at micro- and macro-geographical scales using allozyme polymorphism in many species (analyzed by Nevo 1978, 1983 a, 1988 b, 1995 b, 1998; Nevo and Beiles 1988; Nevo et al. 1984). Local topographic, edaphic, climatic and temporal selection on allozymes has been tested in wild emmer wheat (Nevo and Beiles 1989; Nevo et al. 1982, 1988 a, b, 1991) and wild barley, *Hordeum spontaneum* (e.g. Brown et al. 1980; Nevo et al. 1979, 1986 a,b,c,d). All these results showed that there are significant nonrandom genetic differentiation patterns in both single- and multilocus structures and suggest that allozyme polymorphisms in both wild barley and wild emmer wheat are at least partly adaptive and differentiated primarily by ecological factors, such as alternative soils, topographies, years, or microclimates. All these factors share the common denominator of aridity stress as the major selective cause of genetic differentiation (Nevo 1988 b, 1995 b).

An interesting question is whether DNA polymorphism is also subject to selective forces mirroring the allozymic pattern. This is important because the protein polymorphism observed to be correlated with ecology is produced by the coding sequences of the genome, while DNA-based marker polymorphism is produced by both coding and non-coding genomic regions. On the other hand, the analysis of DNA

polymorphism is considered to be a finer tool than the analysis of protein polymorphism because it permits a much deeper probing into genomic variation (Puterka et al. 1993). However, the correlation between genomic variation and environment remains to be addressed in wild emmer wheat. Although preliminary studies of DNA diversity in wild emmer wheat and wild barley indeed suggested the operation of natural selection (e.g. Flavell et al. 1986; Dawson et al. 1993; Nevo et al. 1995, 1996, 1998; Baum et al. 1997; Pagnotta et al. 1995; Owour et al. 1997; Fahima et al. 1999), much more work still is required to inspect DNA variation across the genome at macro- and micro-geographical scales before any generalizations can be made.

Randomly amplified polymorphic DNA (RAPD) markers are useful for analyzing population genetic structure, because RAPD polymorphism arises from base-pair substitutions or insertion/deletions that modify (or eliminate) the primer site, or from insertions in the genomic sequence that separate the primer sites to a distance that will not permit amplification (Williams et al. 1990). Preliminary results suggest that with respect to both systematics and evolution, RAPD markers reveal high level of polymorphism among, and even within, species that show little RFLP or isozyme polymorphism (Puterka et al. 1993; Whitkus et al. 1994; Latta and Mitton 1997; Papp et al. 1997; Swoboda and Bhalla 1997; Wang et al. 1998).

The objectives of the present study were to detect the DNA polymorphism of wild emmer wheat from stressful microclimatic niches at the Yehudiyya microsite, and to analyze the relationship between ecological microclimates and DNA diversity. A comparison was also conducted between the RAPD polymorphism and the previously studied allozymic differentiation at Yehudiyya (Nevo et al. 1988a).

Materials and methods

Plant materials

Wild emmer wheat, *T. dicoccoides*, 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, grass component in several steppe-like herbaceous formations in the *Quercus ithaburensis* or the *Quercus brantii* open-park forest belts (Zohary 1973).

The Yehudiyya site is in an open oak-park forest of *Q. ithaburensis* in the lower foothills of the Golan Heights, northeast of the Sea of Galilee. Sampling was conducted in pairs in two microclimatic niches: (1) in ***shade***, under the canopies of the oak trees (trees 10–20 m in height, with canopy diameters up to 20 m); (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. The microclimates and plant formations vary significantly in the two microniches (a single-day measurement is given in Table 1). In each separate pair, several plants were sampled first from the shade under the tree canopy, and secondly from the nearby intertree spaces fully exposed to the sun and only a few meters apart. In 1984 and 1985, 137 individuals involving 18 trees were analyzed electrophoretically (Nevo et al. 1988 a). In the present study, 118 individuals (56 from the shade and 62 from the sun) involving 12 trees from the 1985 collection were chosen to germinate the seeds and isolate the DNA from seedling leaves for RAPD analysis.

DNA isolation and PCR amplification

Genomic DNAs were extracted from seedling leaves by the method of Junghans and Metzlaff (1990). DNA amplification for RAPD experiments was conducted as described in Sun et al. (1997) in 20-μl volumes containing 20 ng of template DNA, 0.12 μM of oligonucleotide primer, and 1 × reaction buffer IV, 2 mM $MgCl_2$, 0.5 units of *Taq* DNA polymerase (Advanced Biotechnologies Ltd., UK), and 0.1 mM of dNTP (MBI Fermentas). Reaction mixtures were overlaid with 30 μl of mineral oil. Amplifications were performed in a DNA Thermo-Cycler 480 (Perkin-Elmer) for 45 cycles of 1 min at

Table 1 Ecological backgrounds of sunny and shady microniches at Yehudiyya ($n = 10$ trees), measured in a single day. Data from Nevo et al. (1988 a) with changes

Ecological factors	Sun	Shade	Sun-shade differential
	Mean SE	Mean SE	Mean SE
Temperature:			
air	32.45 ± 0.617	30.35 ± 0.269	2.1* ± 0.515
soil	39.35 ± 0.943	29.37 ± 0.440	9.98* ± 0.922
Soil-air differential	6.9* ± 0.833	−0.98* ± 0.289	7.88* ± 0.393
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	0.25^{ns} ± 0.393
Plant formation	**Sun:** *Psoralea hirsuta*, *Carthamus glaucus*, *Ami majus*, *Olchoria pumilum*, *Eryngium creticum*, *Gundelia tournefortii*, *Lavatera trimestris*, with dense wild cereals	**Shade:** *Ricotia lunaria*, *Tordylium aegyptiacum*, with sparse other species	
Wild emmer	Abundant	Very sparse	

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

94°C, 2 min at 37°C and 2 min at 72°C, and then, a final 7-min extension step at 72°C.

The PCR products were separated by 1.2% agarose-gel electrophoresis, stained with ethidium bromide, and visualized and photographed by an Eagle Eye II image-analysis video system (Stratagene) with ultraviolet light.

Statistics

The RAPD bands were scored as present (1) or absent (0). Each band was regarded as a locus. The data were analyzed for genetic variability by the *POPGENE* program (Version 1.21, Yeh et al. 1997) and for discriminant analysis by *Statistica* (Shenbrot 1993).

Results

In the present study, 20 oligonucleotide primers (Table 2), capable of amplifying reproducible products, were used to characterize 118 individuals from the shady and sunny populations. Two to eight bands from 250 to 1740-bp in size (Table 2) were amplified by each primer. A total of 97 reproducible bands were scored. *T. dicoccoides* is an inbreeding plant, and all individuals are homozygous. All analyses were conducted assuming that each band represents a single locus with two alleles, *absent*, (0) and *present* (1), at each locus. The allele frequencies and gene diversity (*He*) at 84 polymorphic loci in the shade and sun are presented in Table 3 together with a χ^2-test for the homogeneity between the two niches.

Distribution of RAPD polymorphism in the microclimatic niches

Thirteen of the ninety-seven loci proved to be monomorphic in both the shade and sun subpopulations. The remaining 84 loci (87%) were polymorphic: 4 of the 84 loci were polymorphic only in the sun, while not a single locus was polymorphic only in the shade, and 80 loci were polymorphic in both shade and sun. The appearance of only a single band was restricted to one niche: the band of $UBC324_{480}$ did not appear in the shade, but appeared in the sun with an allele frequency of 0.1774. Between the shade and sun microniches, 26 loci varied significantly in allele frequencies at $P = 0.05, 0.01$ and 0.001 levels, with five loci at the $P = 0.10$ level (Table 3). The remaining 53 loci varied non-significantly ($P > 0.10$) between shade and sun.

Test for homogeneity among trees

The simple χ^2 test showed significant differences at 31 loci between shade and sun at the 0.10, 0.05, 0.01, 0.001 levels. These loci then were treated using a log-linear-model analysis to allow for the effects of niches, trees and niche × tree interaction on allele frequencies. It appeared that 24 out of the foregoing 31 loci are still significant when the heterogeneity among trees was taken into account (Table 4). This suggests that microsite differences really exist around the 12 sampled trees.

Table 2 Sequences of primers used in the present study and the amplified fragments produced. OP – primers obtained from Operon, UBC – primers obtained from University of British Columbia

Primer	Sequence (5′ → 3′)	No. of bands[a]	Range of band size (bp)
OPA12	TCGGCGATAG	8	250–980
OPF2	GAGGATCCCT	8	480–1740
OPM18	CACCATCCGT	4	435–1340
UBC116	TACGATGACG	3	440–890
UBC129	GCGGTATAGT	4	600–1100
UBC143	TCGCAGAACG	4	410–1030
UBC144	AGAGGGTTCT	2	750–1050
UBC269	CCAGTTCGCC	2	410–680
UBC285	GGGCGCCTAG	5	400–950
UBC324	ACAGGGAACG	7	400–1330
UBC416	GTGTTTCCGG	5	510–1150
UBC428	GGCTGCGGTA	5	375–880
UBC465	GGTCAGGGCT	6	375–770
UBC479	CTCATACGCG	7	310–1160
UBC493	CCGAATCACT	6	500–1070
UBC546	CCCGCAGAGT	5	620–1330
UBC611	CCATCGTACC	5	650–1160
UBC621	GTCTGCGCTA	4	625–990
UBC635	CTCAGCTCAG	4	400–1500
UBC636	GGGATATCGC	3	760–1240

[a] Number of reproducible and scorable fragments

Genetic differentiation

The estimates of gene diversity, *He* (Nei 1973), for each locus in the two microniches and their means are presented in Table 3. Gene diversities, calculated from those loci with significant allele frequency differences between the two niches (see Table 2), also varied significantly. The average gene diversity over all 97 loci was essentially equal in the sun (0.3053) and the shade (0.3049). The level of RAPD polymorphism (*P*) was higher in the sun than in the shade. These results are partially consistent with the allozymic results (Nevo et al. 1988 a).

Genetic diversity within and between the two microniches (*G*st analysis)

Gene diversity of the wild emmer population at Yehudiyya, subdivided in terms of the shade and sun microclimates, can be analyzed into: the total gene diversity (*Ht*), the mean gene diversity within the population (*Hs*), the average gene diversity between the shady and sunny microniches ($D_{ST} = Ht - Hs$), and the gene diversity between the shade and sun, relative to *Ht*

Table 3 Allele frequency and gene diversity (*He*, Nei 1973) at different polymorphic loci in the shady and sunny subpopulations of *T. dicoccoides* from Yehudiyya

Locus[a]	Allele frequency (present)		χ^2	*He*	
	Shade	Sun		Shade	Sun
OPA12-720	0.4464	0.9032	28.53***	0.4943	0.1748
-500	0.7857	0.9032	3.14[c]	0.3367	0.1748
-470	0.7857	0.7581	0.13	0.3367	0.3668
-370	0.8214	0.8710	0.56	0.2934	0.2448
-300	0.2143	0.2742	0.09	0.3367	0.3980
-250	0.5357	0.8387	12.74***	0.4974	0.2706
OPF2-1740	0.7143	0.8710	4.46*	0.4082	0.2248
-1430	0.2500	0.5806	13.17***	0.3750	0.4870
-1260	0.9286	0.9194	0.04	0.1327	0.1483
-1110	0.8393	0.8871	0.57	0.2698	0.2003
-480	0.9286	0.8548	1.63	0.1327	0.2482
OPM18-1090	1.0000	0.8387	9.87**	0.0000	0.2706
-820	0.1607	0.2742	2.21	0.2695	0.3980
-440	0.2679	0.3226	0.42	0.3922	0.4370
UBC116-890	0.5893	0.6774	0.99	0.4841	0.4370
-670	0.9107	0.8871	0.18	0.1626	0.2003
UBC129-1100	0.1429	0.1935	0.54	0.2449	0.3122
-820	0.4286	0.6129	4.01*	0.4898	0.4745
-750	0.2500	0.2742	0.09	0.3750	0.3980
-600	0.6250	0.6613	0.17	0.4688	0.4480
UBC143-1030	0.6071	0.6613	0.07	0.4770	0.4480
-790	0.7143	0.5645	2.85[c]	0.4082	0.4917
-650	0.4464	0.6290	3.95*	0.4943	0.4667
-410	0.1429	0.4516	13.23***	0.2449	0.4953
UBC144-1050	0.6964	0.7419	0.30	0.4228	0.3829
-750	0.6429	0.5806	0.48	0.4592	0.4870
UBC269-680	0.5357	0.7742	7.47***	0.4974	0.3496
-410	0.2143	0.3871	4.14*	0.3367	0.4745
UBC285-950	0.2679	0.3548	1.03	0.3922	0.4579
-780	0.6607	0.8065	3.23[c]	0.4483	0.3122
-680	0.3929	0.3226	0.63	0.4770	0.4370
-530	0.3214	0.5323	5.33*	0.4362	0.4979
-400	0.8571	0.8710	0.09	0.2449	0.2248
UBC324-***1330***	0.1964	0.4032	5.94*	0.3157	0.4813
-1100	0.6964	0.8065	0.57	0.4228	0.3122
-800	0.3929	0.4677	0.67	0.4770	0.4679
-720	0.7857	0.5000	10.37**	0.3367	0.5000
-580	0.2857	0.1613	1.75	0.4082	0.2706
-480	0.0000	0.1774	10.96***	0.0000	0.2919
-400	0.1429	0.2258	1.33	0.2449	0.3496
UBC416-1150	0.8214	0.8871	1.03	0.2934	0.2003
-1110	1.0000	0.9355	3.74[c]	0.0000	0.1207
-790	0.4464	0.8548	21.90***	0.4943	0.2482
-710	0.7857	0.7581	0.13	0.3367	0.3668
UBC428-710	0.7857	0.7258	0.57	0.3367	0.3980
-580	0.7500	0.7742	0.10	0.3750	0.3496
-500	0.7321	0.4194	11.73***	0.3922	0.4870
-380	0.2143	0.4032	4.88*	0.3367	0.4813
UBC465-770	0.9286	0.7581	6.33*	0.1327	0.3668
-550	1.0000	0.9194	4.72*	0.0000	0.1483
-490	0.5714	0.9355	17.86***	0.4898	0.1207
-400	0.3393	0.4839	2.53	0.4483	0.4995
UBC479-1150	0.5714	0.6935	1.89	0.4898	0.4251
-880	1.0000	0.9194	4.72*	0.0000	0.1483
-820	0.3750	0.3710	0.00	0.4688	0.4667
-710	0.3929	0.5806	4.15*	0.4770	0.4870
-580	0.8393	0.8871	0.57	0.2698	0.2003
-470	0.0893	0.1290	0.47	0.1626	0.2248
-310	0.7143	0.7581	0.29	0.4082	0.3668
UBC493-***1070***	0.6786	0.8548	5.18*	0.4362	0.2482
-970	0.9107	0.9355	0.03	0.1626	0.1207
-830	0.8750	0.9032	0.24	0.2188	0.1748
-760	0.7321	0.8387	2.00	0.3922	0.2706
-650	0.6607	0.6613	0.00	0.4483	0.4480
-500	0.5893	0.3871	4.82*	0.4841	0.4745
UBC546-***1330***	0.3393	0.7581	20.94***	0.4483	0.3668
-1140	0.7143	0.9677	14.62***	0.4082	0.0624
-730	0.2379	0.3387	1.63	0.3922	0.4480
-620	0.1607	0.2581	1.67	0.2698	0.3829
UBC611-1160	0.6964	0.5968	1.27	0.4228	0.4813
-910	0.8929	0.8871	0.01	0.1913	0.2003
-750	0.2857	0.2742	0.02	0.4082	0.3980
-650	0.5893	0.4032	4.08*	0.4841	0.4813
UBC621-**990**	0.6607	0.6129	0.29	0.4483	0.4745
-920	0.3929	0.4839	0.99	0.4770	0.4995
-790	0.8036	0.7581	0.35	0.3157	0.3668
-630	0.5179	0.4839	0.14	0.4994	0.4995
UBC635-1500	0.4286	0.4032	0.08	0.4898	0.4813
-1100	0.4286	0.4194	0.01	0.4898	0.4870
-750	0.1964	0.2742	0.98	0.3157	0.3980
-400	0.7143	0.6290	0.97	0.4082	0.4667
UBC636-1240	0.5179	0.5484	0.11	0.4994	0.4953
-760	0.8571	0.8871	0.24	0.2449	0.2003
Overall 97 loci: Proportion of loci polymorphic	0.8247	0.8660			
Mean				0.3049	0.3053

Monomorphic loci = 13. Significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; c = $P < 0.10$; $df = 1$

[a] Loci marked by a bold font were included in the 25-variable model of discriminant analysis, while those marked by italics and bold fonts were included in the 12-variable model

Table 4 Summary of single-locus tests for significance of the microniche effects on allele distribution in *T. dicoccoides* populations from Yehudiyya

Significance	Number of loci	
	χ^2-test	Log-linear model test
$P < 0.10$	4	4
$P < 0.05$	14	7
$P < 0.01$	2	5
$P < 0.001$	11	8
Total	31	24

Table 5 *Gst* analysis: The partition of RAPD genetic diversity at 32 polymorphic loci (with higher *Gst* differentiation) in *T. dicoccoides* populations within and between the shady and sunny niches at Yehudiyya

Locus	*Ht*	*Hs*	D_{ST}	G_{ST}
$OPA12_{720}$	0.4388	0.3345	0.1043	0.2377
$OPA12_{250}$	0.4299	0.3840	0.0459	0.1068
$OPF2_{1740}$	0.3288	0.3165	0.0123	0.0373
$OPF2_{1430}$	0.4857	0.4310	0.0547	0.1126
$OPM18_{1090}$	0.1483	0.1353	0.0130	0.0877
$UBC129_{820}$	0.4992	0.4822	0.0064	0.0340
$UBC143_{790}$	0.4611	0.4499	0.0112	0.0243
$UBC143_{650}$	0.4972	0.4805	0.0167	0.0335
$UBC143_{410}$	0.4178	0.3701	0.0478	0.1141
$UBC269_{680}$	0.4519	0.4235	0.0284	0.0629
$UBC269_{410}$	0.4205	0.4056	0.0149	0.0355
$UBC285_{780}$	0.3909	0.3803	0.0106	0.0272
$UBC285_{530}$	0.4893	0.4671	0.0222	0.0454
$UBC324_{1330}$	0.4199	0.3985	0.0214	0.0509
$UBC324_{700}$	0.4592	0.4184	0.0408	0.0889
$UBC324_{580}$	0.3471	0.3394	0.0077	0.0223
$UBC324_{480}$	0.1616	0.1459	0.0157	0.0973
$UBC416_{1110}$	0.0625	0.0604	0.0021	0.0336
$UBC416_{790}$	0.4546	0.3712	0.0834	0.1834
$UBC428_{500}$	0.4885	0.4396	0.0489	0.1001
$UBC428_{380}$	0.4268	0.4090	0.0178	0.0418
$UBC465_{770}$	0.2642	0.2497	0.0145	0.0550
$UBC465_{550}$	0.0774	0.0741	0.0033	0.0420
$UBC465_{490}$	0.3716	0.3053	0.0663	0.1784
$UBC465_{400}$	0.4844	0.4739	0.0105	0.0216
$UBC479_{880}$	0.0774	0.0741	0.0033	0.0420
$UBC479_{710}$	0.4820	0.4820	0.0176	0.0353
$UBC493_{1070}$	0.3577	0.3422	0.0155	0.0434
$UBC493_{500}$	0.4997	0.4793	0.0204	0.0409
$UBC546_{1330}$	0.4953	0.4076	0.0877	0.1771
$UBC546_{1140}$	0.2674	0.2353	0.0321	0.1201
$UBC611_{650}$	0.5000	0.4827	0.0173	0.0346
Mean	0.3799	0.3515	0.0286	0.0752
Mean@[a]	0.3156	0.3051	0.0105	0.0335

[a] The @ mean is the average of all 97 loci

($G_{ST} = D_{ST}/Ht$) (Nei 1973). An analysis of the partitioning of gene diversity was done for all 97 loci. Table 5 presents the results of 32 polymorphic loci for which the genetic differentiations were relatively high, and the mean over all 97 loci. For two alleles at a locus, G_{ST} is identical to the F_{ST} (Wright 1946). According to Hartl (1980), the range of 0.05–0.15 for *F*st may be considered to indicate moderate differentiation, while a 0.15–0.25 range may indicate greater differentiation. Therefore, the present data showed great differentiation at four loci ($OPA12_{720}$, $UBC416_{790}$, $UBC465_{485}$ and $UBC546_{1325}$), moderate differentiation at 12 polymorphic loci, and non-negligible differentiation at the other loci since "differentiation is by no means negligible if F_{ST} (here = G_{ST}) is as small as 0.05 or even less" (Wright 1978).

The partitioning of RAPD genetic diversity showed a strong differentiation between the two subpopulations. On the average (Table 5), 92.5% of the total genetic diversity of the 32 polymorphic loci in the two microniches exists within, and 7.5% exists between the two. Over all 97 loci (Table 5), on average, 96.65% of the total genetic diversity exists within, and 3.35% exists between, the two microniches. At the four loci with greatest differentiation ($OPA12_{720}$, $UBC416_{790}$, $UBC465_{485}$ and $UBC546_{1325}$), the contrasting microniches contribute 17.7–23.7% to the genetic differentiation, whereas at the next six loci ($OPA12_{520}$, $OPF2_{1430}$, $UBC143_{410}$, $UBC324_{480}$, $UBC428_{500}$ and $UBC546_{1140}$) the microclimatic contribution was 10–12%. For the allozymes, the average G_{ST} over 16 polymorphic loci was also relatively high (0.2212), suggesting that 22% of the total allozymic diversity exists between the two niches (Nevo et al. 1988 a)

Genetic distance

The unbiased genetic distance (*D*; Nei, 1978) was 0.0292 between the two neighbouring climatic microniches, indicating that genetic differentiation exists between them. Compared with the allozymic results (Nevo et al. 1988 a), this RAPD genetic distance was lower than the average allozymic genetic distance over the 1984 and 1985 collections ($D = 0.115$), but was much higher than the allozymic genetic distance ($D = 0.004$) in the same 1985 collection.

Discriminant analysis

A forward stepwise discriminant analysis was conducted by Wilks' method, which maximizes the overall multivariate *F* ratio of the two climatic microniches, based on a multilocus analysis involving all polymorphic loci. In this study, the RAPD loci were very informative. According to the most-informative 25 loci (marked by a bold font in Table 3) out of the 80 polymorphic loci in both niches, the discriminant function was highly significant [$F_{(25,91)} = 17.186$, $P < 0.00005$], and all the individuals can be classified 100% correctly into the niches from which they were sampled (Table 6 a, Fig. 1). By choosing 12 (marked by the italics and bold fonts in Table 3) of the 25 loci, the discriminant function was also significant [$F_{(12,104)} = 21.900$, $P < 0.00005$, Table 6 b), and 112 (96%) individuals can be correctly assigned to their own niches, whereas only four (7%) plants from the shade were incorrectly assigned to the sun, and two (3%) individuals from the sun were incorrectly assigned to the shade. Even when the discriminant analysis was based on only the two most-informative loci ($OPA12_{720}$ and $UBC416_{790}$), with a significant discriminant function [$F_{(2,114)} = 38.190$, $P < 0.0005$, Table 6 c], 95 (80.5%) individuals also could be correctly classified into the two niches, and only eight plants (14.3%) from the shade and 15 plants (24.2%) from the sun were incorrectly classified into the

Table 6 Classification matrix of discriminant analysis based on 25(a), 12(b) and 2(c) most-informative RAPD loci (rows: observed classification; column: predicted classification)

a: based on 25 loci (see Table 3), Wilks' lambda = 0.1748; $F(25, 91) = 17.186$, $P < 0.00005$

Niche	Percent correct	Shade	Sun
Shade	100.0	56	0
Sun	100.0	0	62
Total	100.0	56	62

b: based on 12 loci (see Table 3), Wilks' lambda = 0.2835; $F(12, 104) = 21.900$, $P < 0.00005$

Niche	Percent correct	Shade	Sun
Shade	92.86	52	4
Sun	98.39	1	61
Total	95.76	53	65

c: based on two loci $OPA12_{720}$ and $UBC416_{790}$. Wilks' lambda = 0.5988; $F(2, 114) = 38.190$, $P < 0.00005$

Niche	Percent correct	Shade	Sun
Shade	85.71	48	8
Sun	75.81	15	47
Total	80.51	63	55

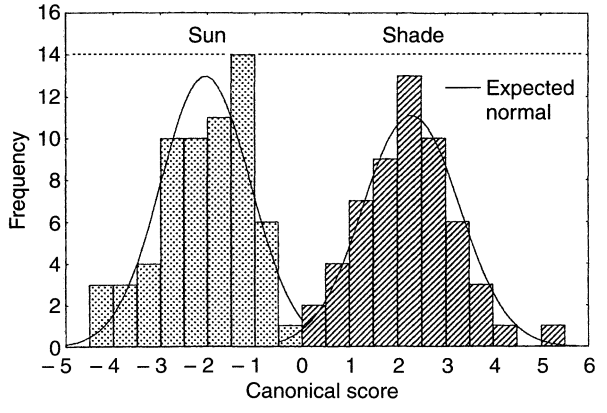

Fig. 1 The histogram of frequencies of canonical scores for the shady and sunny niches according to 25 polymorphic RAPD loci

alternative niche. These results suggest that RAPD differentiation identifies individuals characteristic for their respective niches.

Linkage disequilibrium between RAPD loci

Coefficients (D) of linkage disequilibrium among polymorphic loci were calculated by Weir's (1979) method. For the whole population, as well as for the shady and sunny microniches, 400, 368 and 310 pairs of significant linkage disequilibria were found out of a total of 3315, 2886 and 3310 two-allele/two-locus associations, respectively. A binomial test was conducted with a normal approximation for the binomial distribution. The results showed that the total proportions of significant pairs (12, 13, 9%) were very significantly ($P < 0.00005$, Table 7) higher than expected by chance (5%). A comparison was also conducted for the highly significant ($P < 0.01$) linkage disequilibria in the shaded (119 pairs) and the sunny (102 pairs) microclimatic niches; about 8–9% of these pairs were significant ($P < 0.05$ or 0.01) in both niches, and the remaining associations were significant only in the shade or in the sun. Additionally, many niche-specific linkage disequilibria were found. Table 8 presents a part of the niche-specific linkage disequilibria. For example, for the following pairs, $OPA12_{720A}$-$UBC285_{780A}$ $OPA12_{370A}$-$UBC129_{820A}$, $OPA12_{300A}$-$UBC143_{650A}$, $OPA12_{250A}$-$UBC546_{1140A}$, $UBC143_{1030A}$-$UBC143_{650A}$, $UBC144_{1050A}$-$UBC635_{400A}$, and $UBC269_{680A}$-$UBC465_{770A}$, their linkage disequilibria were significant but with different directions in the two niches.

Table 7 Linkage disequilibrium of the 84 polymorphic RAPD loci for the whole population, and for the shady and the sunny niches

	The whole population	The shade	The sun
Total pairs	3315	2886	3310
Significant LD ($p < 0.05$)	400	368	310
% Total	12	13	9
Binomial test	z = 18.671***	z = 19.107***	z = 11.524***

***$P < 0.00005$

Multilocus analysis

Multilocus structure

The multilocus organization index, proposed by Brown et al. (1980), is based on the observed distribution of the number of heterozygous loci (K) between two randomly chosen gametes. This index, related to the single-locus Simpson index, measures multilocus associations when multiple alleles and many loci are analyzed, combining all paired-locus gametic-phase disequilibria. We calculated multilocus organization indices for the shady and sunny microniches (Table 9). The differences between the expected (σ_k^2) and observed variances (s_k^2) are significant in both microniches. Thus, there is evidence of widespread gametic-phase disequilibrium in the two microniches. The expected variance (σ_k^2) was slightly higher in the sun than in the shade. The observed variance (s_k^2), however, was apparently higher in the shade (49.29) than in the sun (40.84). The standardized index of the intensity of multilocus structure X(2)

Table 8 The niche-specific linkage disequilibrium (D) and normalized linkage disequilibrium (D′) with a χ^2-test for the shady and sunny niches

$Locus_{allele}$	D		D′		χ^2	
	Shade	Sun	Shade	Sun	Shade	Sun
$OPA12_{720A}$-$OPA12_{250A}$	0.1180	0.0005	0.4774	0.0060	12.68**	0.00
$OPA12_{720A}$-$UBC285_{780A}$	− 0.0628	0.0297	− 0.093	0.3393	3.99*	4.00*
$OPA12_{500A}$-$UBC116_{670A}$	0.0523	− 0.0109	0.6431	− 0.0107	11.19**	0.85
$OPA12_{470A}$-$UBC116_{670A}$	0.0523	− 0.0273	0.6431	− 0.0320	11.19**	2.52
$OPA12_{370A}$-$UBC129_{820A}$	− 0.0663	0.0468	− 0.0346	0.4167	6.86**	5.10
$OPA12_{300A}$-$UBC116_{670A}$	0.0191	− 0.0658	0.2353	− 0.2129	1.50	13.47**
$OPA12_{300A}$-$UBC143_{650A}$	0.0651	− 0.0918	0.3864	− 0.2107	5.70*	11.26**
$OPA12_{250A}$-$UBC546_{1140A}$	− 0.0612	0.0271	− 0.0816	0.8667	4.14*	10.75**
$OPF2_{1430A}$-$OPF2_{1110A}$	− 0.0313	0.0656	− 0.1049	0.6545	2.16	10.93**
$UBC116_{890A}$-$UBC621_{990A}$	0.0214	− 0.0926	0.0953	− 0.0838	0.47	11.42**
$UBC116_{890A}$-$UBC621_{630A}$	0.0877	− 0.0536	0.3623	− 0.0383	7.13**	3.26
$UBC116_{670A}$-$UBC143_{1030A}$	0.0006	0.0747	0.0078	0.7455	0.00	15.41**
$UBC116_{670A}$-$UBC493_{830A}$	0.0424	− 0.0109	0.5216	− 0.0111	11.33**	0.85
$UBC129_{820A}$-$UBC636_{700A}$	− 0.0867	0.0161	− 0.1590	0.0680	10.22**	0.27
$UBC143_{1030A}$-$UBC143_{650A}$	− 0.0746	0.0840	− 0.0549	0.3751	5.29*	8.38**
$UBC144_{1050A}$-$UBC635_{400A}$	0.0561	− 0.0957	0.2750	− 0.0812	12.72**	4.09*
$UBC269_{680A}$-$UBC465_{770A}$	− 0.0332	0.0583	− 0.0575	0.3333	3.73*	6.57**
$UBC285_{530A}$-$UBC621_{790A}$	0.0631	− 0.0486	0.4000	− 0.0693	6.48**	3.21
$UBC285_{400A}$-$UBC636_{760A}$	0.0689	− 0.0146	0.5625	− 0.0148	17.72**	1.17
$UBC324_{1330A}$-$UBC465_{770A}$	0.0140	− 0.0960	0.2115	− 0.1805	1.05	12.95**
$UBC324_{800A}$-$UBC465_{770A}$	0.0102	− 0.1126	0.1538	− 0.1826	0.37	17.23**
$UBC324_{700A}$-$UBC635_{1100A}$	− 0.0867	0.0323	− 0.0473	0.1325	10.22**	1.06
$UBC416_{1150A}$-$UBC416_{790A}$	− 0.0989	− 0.0003	− 0.0537	− 0.0003	15.10**	0.00
$UBC416_{790A}$-$UBC416_{710A}$	− 0.0115	0.0617	− 0.0202	0.4969	0.18	10.36**
$UBC428_{580A}$-$UBC465_{770A}$	0.0536	− 0.0224	0.8077	− 0.0219	12.92**	0.97

*·** = $P < 0.05$, 0.01, respectively

was 1.79 in the shade and 1.29 in the sun. These values of X(2) indicate a slightly higher degree of multilocus association in the shade than in the sun.

Multilocus analysis of population subdivision

This analysis can partition the structure, both of multilocus associations among and within several populations, into its components (Brown and Feldman 1981). These components are measured by their contributions to the variance in terms of the number of heterozygous loci in two randomly chosen gametes. The single-locus components represent the average and the variation among (sub)populations in gene diversity and the variance among (sub)populations in allele frequency. The two-locus components include the mean and variance of disequilibria, the covariance of allele frequencies over (sub)populations, and various interactions. This analysis was applied to the present RAPD data. The results are presented in Table 10. Of the total observed variance in the number of heterozygous loci for pairs of random gametes in the mixed pool, i.e. the whole Yehudiyya population, the single-locus components accounted for 51%. The variance of mean disequilibrium (MD = 16.6699) and the variance of disequilibrium (VD = 10.4296) were very high, and MD accounted for 95% of the two-locus effects (MD + WC + AI). This indicates that disequilibria are not only very high in both microniches, but also differ between the two microniches. The Wahlund effect (WC), i.e. the covariance of allele frequency, and the interaction between MD and WC (AI), both were very low. Although the VD was high, the covariance of interaction (CI) was apparently low. This suggests that founder effects might not be important. Similarly, population subdivision might also be low since both WC and AI were low. Hence, multilocus associations of the two subpopulations arise mainly from natural selection, and not by chance from population subdivision or founder effects.

Table 9 Multilocus analysis for the shady and sunny microniches at Yehudiyya. Analysis was conducted by the method of Brown et al. (1980)

Microniche	Shade	Sun
Polymorphic loci	80	84
Expected var. of K[a]	17.6577	17.8024
Upper limit of 95%	24.1641	24.0395
Estimate of var. of K	49.2901*	40.8385*
X(2)[b]	1.7914	1.2940

[a] K = number of heterozygous loci in two randomly chosen gametes
[b] The measures of multilocus structure. The X(2) value for shade + sun was 1.4321
*$P < 0.05$

Table 10 Multilocus analysis of population subdivision for the *T. dicoccoides* population at Yehudiyya

Source of variance	Value of variance
Single-locus effect:	
Mean gene diversity (MH)	17.7337
Variance of diversity (VH)	0.3228
Wahlund's effect (WH)	0.2130
Total	18.2695
Two-locus effect:	
Mean disesquilibrium (MD)	16.6699
Wahlund's effect (WC)	0.8453
Interaction between MD and WC (AI)	0.0361
Variance of dissequilibrium (VD)	10.4296
Covariance of interaction (CI)	−0.0199
σ^{2a}_{k}, average variance of K	44.8494
${}^{T}\sigma^{2}_{k}$, variance of K in mixed population	35.8209

[a] σ^{2}_{k} = MH + MD + AI + VD + CI
[b] ${}^{T}\sigma^{2}_{k}$, = MH + VH + WH + MD + WC + AI
k = the number of these loci (K = 0, 1, … m) which are different (heterozygous) when two random gametes are compared at these loci

Discussion

Previous allozyme analysis already had demonstrated single and multilocus genetic differentiation in wild emmer wheat and wild barley at both the macro- and micro-geographical levels (reviewed in Nevo 1983 a, b, 1988 b, 1995 b). Those analyses involved environmental effects, such as microclimatic, edaphic selection, topographical and temporal effects, and found significant genetic differentiation between ecologically different sites, as well as higher allozymic polymorphism in the more variable and stressful environments than in the less variable and stressful ones. Further DNA analysis subsequently showed a DNA diversity pattern similar to that of the allozymic differentiation (Nevo 1988 b, 1998; Nevo et al. 1996, Owour et al. 1997; Fahima et al. 1999). These results suggest that natural selection may play an important role in the genetic differentiation of natural (sub)populations of wild emmer wheat and wild barley, not only at the protein level but also at the DNA level.

In the Yehudiyya climatic microniches, significant genetic allozyme differentiation in single-, two- and multilocus structures was found in wild emmer wheat between neighbouring climatic niches, and higher polymorphism existed in the more unpredictable and more variable sunny microniche. These results suggested 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 (Nevo et al. 1988 a).

At the DNA level, the present results of genetic differentiation between the microclimatic niches at the Yehudiyya site suggest that genetic diversity and differentiation in allele frequency at the single-locus level, but mainly at the multilocus level, also are subject to microclimatic selection. This is indicated by the following results: (1) single-locus differentiation (Tables 3, 5) and discriminant analysis, which suggested that these single-locus differentiations were informative enough to characterize the individuals of the two neighboring niches and to 100% correctly distinguish these individuals according to multilocus analysis (Table 6, Fig. 1); (2) much more significant linkage disequilibria than those expected by chance (Table 7), and a significant microclimatic niche-effect on the significance of the two-locus linkage disequilibria, and niche-specific linkage disequilibrium (Table 8); (3) a significant structure of the multilocus associations (Table 9), and a slightly higher degree of multilocus association in the shady, compared with that in the sunny, niche; (4) multilocus analysis of population subdivision indicated that very high and different disequilibria in the two microniches mainly arise from natural selection, and not by chance population subdivision or founder effects (Table 10). As in the allozymic polymorphism (Nevo et al. 1988 a), these results parallel the sharp microgeographical differentiation reported for other plants such as wild barley, *H. spontaneum* (Nevo et al. 1981, 1983, 1986 d, 1997), wild oat, *Avena barbata* (Hamrick and Allard 1972; Hamrick and Holden 1979), and *Litaris cylindracea* (Schall 1974, 1975). According to the allozymic results, Nevo et al. (1988 a) suggested that genetic differentiation at the Yehudiyya microsite is affected at least partly by the aridity stress faced by the various life cycles of wild emmer. Presumably, its DNA differentiation also is related to aridity stress.

Since the population size of wild emmer wheat at Yehudiyya involves many thousands of individuals, the effect of a small-size population cannot explain either the allozymic pattern (Nevo et al. 1988 a) or the DNA pattern discovered here. The genetic neighbourhood effect was about 5 m in diameter in Yehudiyya (Golenberg 1986). The mosaic oak tree structure of the present experimental design and the 12 repetitions may protect our results, at least partly, from genetic neighbourhood effects.

According to the allozymic markers, migration in the Yehudiyya population was estimated to be low, about 1.25 m per generation (Golenberg 1986). This result suggests that gene flow is not a predominant force in the structuring of genetic variation in the wild emmer wheat population at this site, and that selection overrides migration. Clearly, when analyzing populations of *T. dicoccoides*, both scale and time must be considered (Golenberg 1986). Nevo et al. (1988 a) also pointed out that the sun-shade allozymic differentiation is unlikely to be the result of stochastic processes. Rather reflects microclimatic niche differentiation in a relatively small area of less than 1000 m^2, and over only a few meters between sun and shade. Based on the RAPD markers, and using G_{ST} (Nei 1973) instead of

F_{ST} (Wright 1946), because the two measures are identical for two alleles/locus, the potential migration rate Nm of 7.21 individuals/generation [$Nm = 0.25 (1 - F_{ST})/F_{ST}$ (Slatkin 1985), G_{ST} was 0.0335 for the overall 97 loci in Table 5] appears to be relatively high, as estimated from RAPDs. A high rate of gene flow may counteract selection and rapidly swamp out interpopulational differentiation in such a selfing system (Golenberg 1986). However, the present analyses had already indicated that the significant RAPD differentiation between the two microclimatic niches is mainly due to natural selection not only at a single locus but also at a multilocus level. On the other hand, if there were not to be strong microclimatic selection in such a small area of less than a few meters, and over a few meters between the sun and the shade, such differentiation might not have been maintained, because a surprisingly small amount of migration is sufficient to swamp out the differentiation that arises from the random drift of non-selection genes (Lewontin 1974). Jain and Bradshaw (1966), McNeilly (1968), Waser et al. (1982), and Hilbish and Koehn (1985) have also presented evidence for selection-generated differentiation in populations of plants and animals in which gene flow was demonstrably, or reasonably assumed to be, high. Therefore, we conclude that both the allozyme and RAPD evidence suggests that selection overrides migration.

These DNA data can not be explained by the hypothesis that molecular diversity is neutral, and that its fate is determined by mutational input and the random extinction or fixation of alleles, as suggested by the neutral theory of molecular evolution (Kimura 1983). Natural selection effects were indicated for specific two-locus linkage disequilibrium for each microclimatic niche, as well as for two-locus subdivision and multilocus association. The aridity index presumably plays a major differentiating role here, as in other sites (Nevo 1995 b). The different genetic compositions of the shady and the sunny microniches may be regarded as an adaptive strategy for increasing fitness in their alternative ecologies. This pattern is in accordance with niche variation (Van Valen 1965), as reflected in their higher P values in the sun. This result supports the regional and global patterns (Nevo 1988 b), where allozymic diversity and DNA differentiation generally increase with increasing spatiotemperal ecological heterogeneity and unpredictability. As in other cases, the DNA patterns were similar to the allozymic patterns (e.g. Nevo et al. 1996, Owuor et al. 1997; Nevo 1998), and the present DNA results, parallel to the allozymic differentiation, meet the theoretical prediction that the existence of a protected polymorphism is more likely in a more heterogeneous environment (Karlin 1982). All these results suggest that the DNA differentiation observed here may be mainly due to natural selection, as was earlier shown by allozymic polymorphism (Nevo et al. 1988 a).

In the present study, higher P value was found in the sunny microniche than in the shade. The two-locus pairs with significant linkage disequilibrium, as well as the intensity of multilocus associations, were higher in the shade than in the sun. This two-locus pattern is not consistent with the previous allozymic results (Nevo et al. 1988 a). We hypothesize that the shady environment may introduce additional stresses, such as dim light and even minimal soil water availability, due to competition with the oaks.

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