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Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides,* in Israel

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Abstract Diversity in 20 microsatellite loci of wild emmer wheat, *Triticum dicoccoides*, was examined in 15 populations (135 genotypes) representing a wide range of ecological conditions of soil, temperature, and water availability, in Israel and Turkey. An extensive amount of diversity at microsatellite loci was observed despite the predominantly selfing nature of this plant species. The 20 Gatersleben wheat microsatellites (GWM), representing 13 chromosomes of genomes A and B of wheat, revealed a total of 364 alleles, with an average of 18 alleles per GWM marker (range: 5–26). The proportion of polymorphic loci per population averaged 0.90 (range: 0.45–1.00); genic diversity, *He*, averaged 0.50 (range 0.094–0.736); and Shannon's information index averaged 0.84 (range 0.166–1.307). The coefficients of genetic distance between populations were high and averaged *D*=1.862 (range 0.876–3.320), an indication of sharp genetic divergence over short distances. Interpopulation genetic distances showed no association with geographic distance between the population sites of origin, which ruled out a simple isolation by distance model. Genetic dissimilarity values between genotypes were used to produce a dendrogram of the relationships among wild wheat populations by the unweighted pair-group method with arithmetic averages (UPGMA). The results showed that all the wild emmer wheat populations could be distinguished. Microsatellite analysis was found to be highly effective in distinguishing genotypes of *T. dicoccoides,* originating from diverse ecogeographical sites in Israel and Turkey, with 88% of the 135 genotypes correctly classified into sites of origin by discriminant analysis. Our present microsatellite results are non-random and in agreement with the previously obtained allozyme and RAPD patterns, although the genetic-diversity values obtained with microsatellites are much higher. Significant correlates of microsatellite markers with various climatic and soil factors suggest that, as in allozymes and RAPDs, natural selection causes adaptive microsatellite ecogeographical differentiation, not only in coding, but most importantly in non-coding genomic regions. Hence, the concept of "junk DNA" needs to be replaced by at least partly regulatory DNA. The obtained results suggest that microsatellite markers are useful for the estimation of genetic diversity in natural populations of *T. dicoccoides* and for the tagging of agronomically important traits derived from wild emmer wheat.

Keywords Genetic diversity · Microsatellite markers · Wild emmer wheat · *Triticum dicoccoides*

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Introduction

Wild emmer wheat, *Triticum dicoccoides*, is the tetraploid progenitor of cultivated hexaploid bread wheat. It has been the focus of many genetic, ecological, physiological and cytogenetic studies. The rich genetic diversity of wild emmer wheat for multiple disease resistances, agronomic traits of economic significance, and environmental adaptations has been reviewed previously (Nevo 1983, 1989, 1995, 2001). Populations of wild wheat are geographically structured and are predictable by ecological (climatic, soil, and biotic) factors and molecular markers. Of special relevance to the present paper are the previous studies scoring the allelic diversity at allozyme and RAPD loci in wild emmer wheat populations from Israel and Turkey (Nevo et al. 1982; Nevo and Beiles 1989; Fahima et al. 1999). From the distribution and frequencies of the defined alleles, it was concluded that natural selection was responsible for some of the differences between populations residing in localities with different climates and soil types, in both coding (allozymes) and partly coding

(RAPDs) genomic regions. The present study explored the diversity in the largely non-coding microsatellite markers across 13 chromosomes, i.e. most of the wheat genome, and its relationship with the allozymic and RAPD diversity recorded earlier in these populations.

Microsatellites or simple sequence repeats (SSRs) are tandem repeats of short (2–6 bp) DNA sequences (Litt and Luty 1989). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing diversity in the number of repeating units. This kind of polymorphism at specific loci is easily detected using specific primers in the flanking regions of such loci and subsequent amplification via the polymerase chain reaction (Litt and Luty 1989; Weber and May 1989). The high level of polymorphism, combined with a high interspersion rate, makes them an abundant source of genetic markers. Microsatellites are one of the most promising molecular-marker types able to identify or differentiate genotypes within a species. Their co-dominant inheritance, high level of polymorphism and easy handling make them extremely useful for many applications. Microsatellites may provide a source of abundant quantitative variability based on mutations that are frequent, site-specific and reversible, yet seldom substantially deleterious (Kashi et al. 1997; King et al. 1997; Kashi and Soller 1999; King and Soller 1999). Microsatellites may be evolutionarily significant, equipping genomes and individual genes with adjustable 'turning knobs' for efficient adaptation (Kashi et al. 1997; Caudrado and Schwarzacher 1998; King and Soller 1999). Wheat microsatellites have been successfully used for the construction of genetic linkage maps of wheat (Röder et al. 1998), for detection of genetic diversity (Fahima et al. 1998; Li et al. 2000a,b,c), and for mapping of agronomically important genes (Korzun et al. 1998; Chague et al. 1999; Peng et al. 1999, 2000a,b, 2000c).

In previous papers we demonstrated the application of wheat microsatellites for various studies of *T. dicoccoides*. These included the identification of wild wheat accessions highly resistant to stripe rust (Fahima et al. 1998), mapping of the stripe rust resistance genes *YrH52* (Peng et al. 1999, 2000a) and *Yr15* (Chague et al. 1999; Peng et al. 2000b), construction of genetic linkage maps in tetraploid wheat (Peng et al. 2000b), and microscale differentiation of *T. dicoccoides* sub-populations (Li et al. 2000a,b,c). In the study reported here, we demonstrated the application of Gatersleben wheat microsatellite (GWM) markers for the estimation and differentiation of genetic diversity at the macro-geographic scale among 15 populations of *T. dicoccoides*. These were collected in Israel and Turkey from various locations covering an extensive ecogeographical spectrum of *T. dicoccoides* and representing a wide range of abiotic and biotic ecological conditions of soil, temperature, altitude, vegetation, various pathogens, and water availability.

Materials and methods

Plant material and the ecological background of *T. dicoccoides*

Wild emmer wheat, *T. dicoccoides* (genomic constitution AABB), is the tetraploid, predominantly self-pollinated, wild progenitor from which modern tetraploid and hexaploid cultivated wheats were derived (Zohary 1970). Wild emmer is distributed over the Near East Fertile Crescent in Israel, Jordan, Lebanon, Syria, eastern Turkey, northern Iraq, and western Iran (Harlan and Zohary 1966). The center of distribution and diversity of *T. dicoccoides* is found in the catchment area of the upper Jordan Valley in Israel and its vicinity (Nevo and Beiles 1989). Wild emmer ranges over a wide altitudinal amplitude. Robust, early maturing phenotypes grow on the winter-warm slopes facing the Sea of Galilee, as low as 100-m below sea level. More slender and late-flowering types occur in higher and cooler elevations, reaching 1600 m on Mount Hermon (Zohary 1970; E. Nevo, personal observations). In this study we examined 135 *T. dicoccoides* accessions representing 15 populations collected from various locations, (Amiran et al. 1970) in Israel and Turkey, which represent a wide range of ecological conditions of soil, temperature, altitude, and water availability. The populations used in this work, along with their geographic origin and climatic conditions, are listed in Table 1. A full description of these populations can be found in Nevo et al. (1982) and Nevo and Beiles (1989).

Table 1 Geographic and climatic data for 15 populations of wild emmer wheat, *T. dicoccoides*, in Israel and Turkey. Symbols of variables: **Geographic:** Ln=longtitude (decimals); Lt=latitude (decimal); Al=Altitude (m); **Temperature:** Tm=mean annual temperature; Ta=mean August temperature; Tj=mean Jannuary temperature; Td=seasonal temperature difference; Tdd=day-night temperature difference; Trd=mean number of tropical days; **Water availability:** Rn=mean annual rainfall (mm); Rd=mean number of rainy days; Huan=mean annual humidity; Hu14=mean humidity at 14:00; Dw=mean number of dew nights in summer; Ev=mean annual evaporation; Rv=mean interannual variability of rainfall; Rr=mean relative variability of rainfall; **Edaphic:** So=soil type; 1=terra rossa; 2=rendzina; 5=basalt

No.[a]	Population	Ln	Lt	Al	Tm	Ta	Tj	Td	Tdd	Rn	Rd	Hu14	Huan	Dw	Trd	Ev	So	Rv	Rr
1	**Mt. Hermon**	35.73	33.30	1300	11	21	3	18	6	1400	66	48	60	60	0	150	1	30	20
7	**Yehudiyya**	35.70	32.93	200	19	27	11	16	12	550	47	42	58	58	100	160	5	38	25
8	**Gamla**	35.74	32.88	200	19	26	9	17	12	470	50	43	58	58	60	155	5	39	26
9	**Rosh Pinna**	35.52	32.95	700	18	25	9	16	10	697	50	48	58	50	35	150	1	35	22
11	**Tabigha**	35.53	32.90	0	24	32	15	17	10	436	45	45	57	58	120	160	5	39	25
16	**Mt. Gilboa**	35.42	32.50	150	21	28	12	16	12	400	44	43	58	40	160	165	1	34	24
17	**Mt. Gerizim**	35.28	32.20	800	17	23	8	15	9	700	47	45	60	42	0	155	1	38	25
18	**Gitit**	35.40	32.10	300	21	29	13	16	12	360	39	39	55	25	100	170	1	38	24
19	**Kokhav Hashahar**	35.34	31.95	600	20	28	12	16	12	400	40	45	59	30	25	165	1	38	22
23	**J'aba**	35.08	31.67	660	17	25	9	15	9	500	41	49	62	57	30	155	1	35	21
24	**Amirim**	35.45	32.93	600	15	24	8	16	8	850	61	48	60	53	13	153	1	35	23
28	**Bet-Oren**	35.03	32.73	400	17	24	11	13	8	700	55	59	69	80	0	142	1	25	19
30	**Bat-Shelomo**	35.02	32.60	75	20	26	13	13	10	650	55	58	68	77	30	150	2	24	20
33	**Givat Koach**	34.92	32.03	75	20	26	12	14	12	540	46	50	64	65	105	160	1	32	26
36	**W. Diyarbakir**	39.63	37.89	850	13	27	2	25	–	546	65	–	46	–	–	–	5	–	–

[a] Population numbers are according to Nevo and Beiles (1989)

Wheat microsatellites (GWM) analysis

DNA samples were isolated from whole-wheat grains as described by Plaschke et al. (1995). The microsatellite primers used are as described by Röder et al. (1995, 1998) and Plaschke et al. (1995, 1996). Twenty primer pairs representing Gatersleben wheat microsatellites (GWM) of 13 chromosomes that amplify the expected fragments (according to sequence data) in *T. aestivum* cv 'Chinese Spring' ('CS') were chosen for the analysis. GWM designation, fragment sizes in 'CS', range of allele size, and chromosome-arm location of the amplified loci are presented in Table 2. PCR amplifications were performed as described in Röder et al. (1995). The PCR-amplified fragments were detected by an automated laser fluorescence (ALF) sequencer (Pharmacia). To allow this, one primer of each pair was labelled at the 5′ end with fluorescein. ALF gel running conditions are as described by Plaschke et al. (1995). Fragment sizes were calculated in the computer program Fragment Manager (Pharmacia) by comparison with internal size standards, which were added to each lane in the loading buffer.

Statistical analysis

The programs *POPGENE* (Yeh et al. 1997), *DISPAN* (T. Ota, Pennsylvania State University, Pa, USA) and *STATISTICA* (Statsoft 1996) were used to analyze the data. *POPGENE* was used to compute allele frequencies, genetic polymorphism (*P*-5%), Nei's gene diversity (*He*), and Shannon's information index (*I*). *DISPAN* was used to compute Nei's standard genetic distance coefficients (*D*, Nei 1972), to construct a UPGMA dendrogram (Sneath and Sokal 1973) and to perform bootstrap tests (Felsenstein 1985) for the obtained trees (1000 replications). *STATISTICA* was used to conduct discriminant analysis and stepwise multiple regression (MR), with eco-geographical variables as the independent variables, to find out the best predictors of *P*, *He*, Shannon's Information Index, allele number per locus and representative allele frequencies at each of the 20 polymorphic microsatellite loci.

Results

Wheat microsatellites in wild emmer wheat

Wild emmer wheat collections included a total of 135 accessions, of which 126 were from Israel and nine from Turkey (22 km west of Diyarbakir). The collections from Israel were from 14 locations, namely Mt. Hermon, Yehudiyya, Gamla, Rosh-Pinna, Tabigha, Mt. Gilboa, Mt. Gerizim, Gitit, Kokhav Hashahar, J'aba, Amirim, Bet-Oren, Bat-Shelomo and Givat Koach (Table 1). The geographic distribution of 14 wild wheat populations is depicted in Fig. 1. Only one of the tested populations is not shown in Fig. 1 since it was collected in southern Turkey (see Fig. 1 in Nevo and Beiles 1989). The exact location (name, longtitude, latitude, and altitude) of each of the 15 populations examined here is recorded in Table 1, together with some climatic data. In all, 364 microsatellite alleles were detected with 20 GWM markers, representing 20 microsatellite loci, located on 13 different chromosomes and 20 different chromosome arms of the A and B genomes of wheat (Table 2). All 20 GWM markers yielded polymorphic fragments among the 135 *T. dicoccoides* accessions used in this study. The number of alleles per GWM ranged from 5 to 26 (Table 2). On average, 18.2 alleles were detected per locus. The most polymorphic microsatellite was GWM218 with 26 alleles. The sizes of the 26 alleles ob-

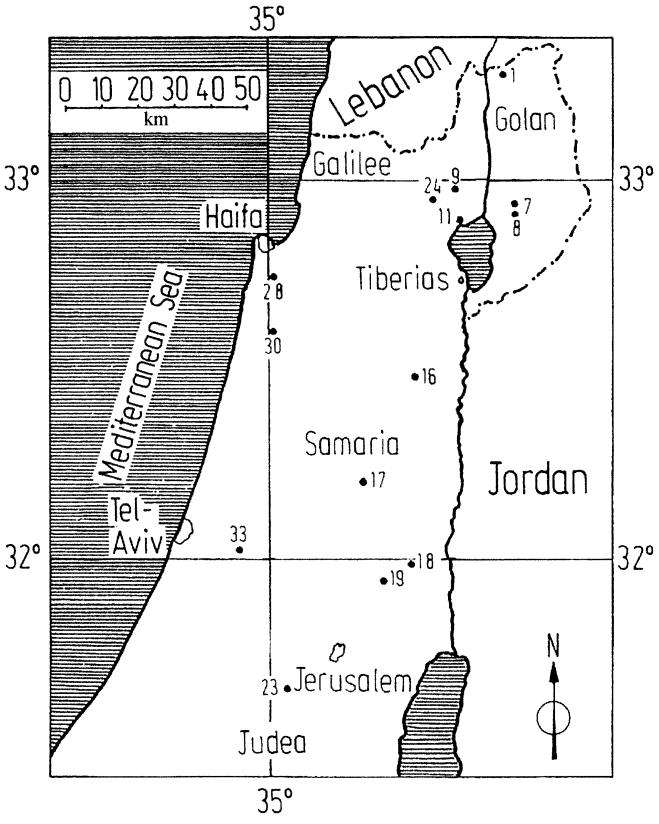

Fig. 1 Geographic distribution of the 14 tested populations of wild emmer wheat, *T. dicoccoides*, in Israel. For names of the numbered populations see list in Table 1. The numbers of populations are according to Nevo and Beiles (1989)

Table 2 Wheat microsatellites, chromosomal-arm locations, number of alleles, and range of allele size for the GWM markers employed. GWM marker designation and chromosomal location are according to Röder et al. (1995, 1998), Plaschke et al. (1995, 1996), Peng et al. (2000b)

Designation	Chromosomal location	Number of alleles	Fragment size in 'CS' (bp)	Range of allele size (bp)
GWM18	1BS	17	186	181–217
GWM60	7AS	18	211	171–221
GWM95	2AS	14	121	107–135
GWM99	1AL	20	119	93–179
GWM120	2BL	20	139	119–171
GWM154	5AS	20	102	99–153
GWM155	3AL	21	141	134–196
GWM169	6AL	21	196	186–230
GWM186	5AL	20	140	102–186
GWM218	3AS	26	153	130–216
GWM219	6BL	15	181	142–190
GWM234	5BS	21	241	208–256
GWM251	4BL	18	103	57–119
GWM265	2AL	5	200	166–184
GWM340	3BL	21	132	97–167
GWM361	6BS	10	126	135–153
GWM389	3BS	24	130	84–172
GWM400	7BS	13	139	128–164
GWM408	5BL	22	176	144–202
GWM577	7BL	18	133	136–182

Table 3 Summary of genetic diversity, based on 20 microsatellite loci in 15 populations of wild emmer wheat, *T. dicoccoides*, in Israel and Turkey

No.[a]	Population[b]	Polymorphic per population *P*[c]	Genetic diversity *He*[d] (SE)	Shannon's information index[e] (SE)
1	Mt. Hermon	1.00	0.736 (0.142)	1.307 (0.362)
7	Yehudiyya (sun/shade)	0.90	0.506 (0.202)	0.761 (0.317)
8	Gamla	0.70	0.288 (0.262)	0.439 (0.412)
9	Rosh-Pinna	0.90	0.473 (0.206)	0.723 (0.348)
11	Tabigha	1.00	0.636 (0.124)	1.059 (0.277)
16	Mt. Gilboa	0.80	0.381 (0.241)	0.632 (0.410)
17	Mt. Gerizim	1.00	0.634 (0.153)	1.086 (0.332)
18	Gitit	0.95	0.648 (0.194)	1.129 (0.395)
19	Kokhav Hashahar	0.95	0.494 (0.205)	0.801 (0.375)
23	J'aba	1.00	0.569 (0.154)	0.968 (0.304)
24	Amirim	1.00	0.618 (0.193)	1.097 (0.402)
28	Bet-Oren	0.45	0.094 (0.155)	0.166 (0.270)
30	Bat-Shelomo	0.95	0.573 (0.197)	0.960 (0.376)
33	Givat Koach	0.85	0.249 (0.185)	0.421 (0.309)
36	w. Diyarbakir, 22 km	1.00	0.603 (0.225)	1.049 (0.449)
Mean		0.90	0.500 (0.198)	0.840 (0.355)

[a] Population numbers are according to Nevo and Beiles (1989)
[b] Sample size of nine accessions per population
[c] *P* =Proportion of polymorphic loci
[d] *He* =Genic diversity, equivalent to the expected heterozygosity under panmixia (Nei 1978)
[e] Shannon's information index (Shannon and Weaver 1949)

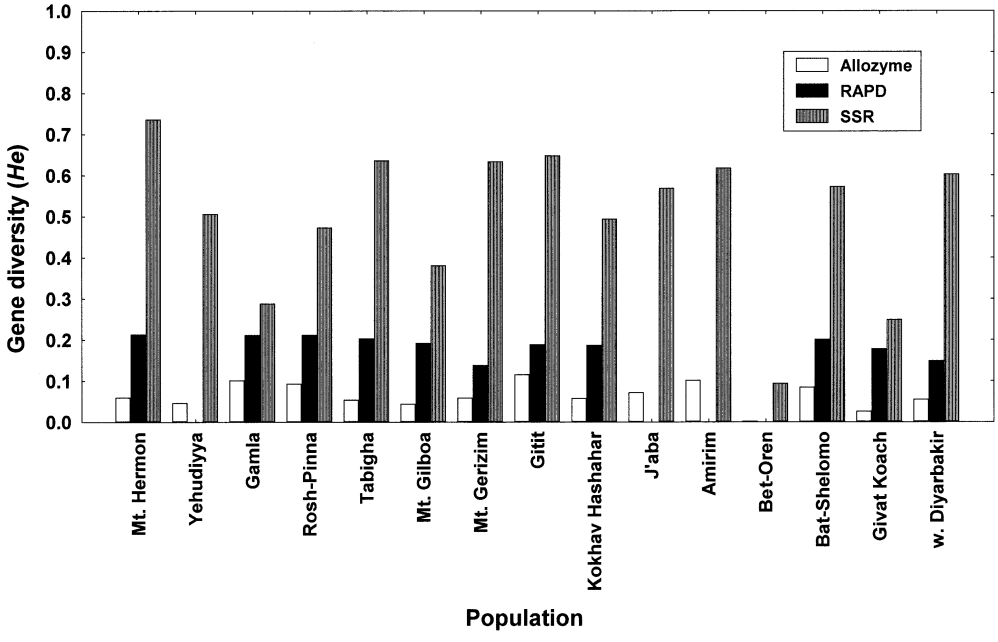

Fig. 2 Gene diversity (*He*, Nei 1978) profiles of microsatellite, RAPD and allozyme loci in 15 wild emmer wheat populations from Israel and Turkey

served ranged from 130 to 216 bp. GWM218 is a perfect microsatellite with a $(CT)_{18}$ motif in 'CS' (Korzun et al. 1997). Hence, since the size of the amplified fragment in 'CS' obtained in this study was 156 bp, it is assumed that the number of dinucleotide repeats of the repetitive motif of GWM218 ranged from 5 to 48 in the *T. dicoccoides* collection employed.

Microsatellite genetic diversity

The genetic data for each of the 15 populations of *T. dicoccoides* are summarized in Table 3. Mean levels of the proportion of polymorphic loci *P* (5%), the genetic diversity *He* (Nei 1978), and the Shannon's information index (Shannon and Weaver 1949) of the 15 populations of wild emmer wheat were 0.90, 0.50, and 0.84 respectively. The range of diversity in *He* between the wild wheat populations was large, 0.094–0.736. The highest value of *He* (0.736) was obtained for the high-altitude and arid-cold steppic population of Mt. Hermon, which represents one of the most stressful xeric-cold habitats in Israel. By contrast, low levels of diversity, with *He* values of 0.094 and 0.249, characterized the isolated small populations from Bet-Oren on Mt. Carmel and Givat Koach near Tel Aviv on the coastal plain, respectively.

Microsatellite versus RAPD and allozyme genetic-diversity profiles

The genetic-diversity profiles, obtained in this study with microsatellite markers, were compared with those obtained previously with allozyme (Nevo et al. 1982; Nevo and Beiles 1989) and RAPD (Fahima et al. 1999) markers (Fig. 2). All populations studied here showed a

Table 4 Coefficients of genetic distance (*D*; Nei 1972) among 15 populations of *T. dicoccoides* in Israel and Turkey

Population[a]	1	7	8	9	11	16	17	18	19	23	24	28	30	33
1. Mt. Hermon														
7. Yehudiyya	1.334													
8. Gamla	1.508	1.674												
9. Rosh-Pinna	1.319	1.896	2.274											
11. Tabigha	1.198	0.889	1.798	2.100										
16. Mt. Gilboa	1.649	1.321	2.860	2.246	2.011									
17. Mt. Gerizim	0.996	1.827	1.425	1.194	1.162	2.350								
18. Gitit	1.371	1.482	1.531	1.765	1.513	2.035	1.873							
19. Kokhav Hashahar	2.313	2.678	2.026	3.073	2.219	3.211	1.912	1.761						
23. J'aba	1.627	1.719	2.425	2.092	1.639	2.106	2.094	1.233	1.919					
24. Amirim	1.208	1.489	1.847	1.225	1.055	1.492	1.400	1.434	2.167	0.876				
28. Bet-Oren	1.502	2.845	2.157	2.255	3.320	2.081	2.246	2.217	2.727	1.154	1.096			
30. Bat-Shelomo	1.649	1.691	1.833	1.502	1.602	1.550	1.833	1.736	1.315	2.149	1.688	2.776		
33. Givat Koach	2.003	2.206	2.592	2.144	2.417	2.349	1.973	2.012	2.089	1.586	2.219	1.896	2.160	
36. W. Diyarbakir	1.654	1.780	2.735	1.951	1.996	1.122	2.106	1.956	2.547	1.718	1.559	1.712	2.058	1.225

[a] Population ID number is according to Nevo and Beiles (1989); average distance=1.862, range=0.876–3.320

higher proportion of polymorphic loci (*P*) and higher genetic diversity (*He*) values for microsatellite loci than for allozyme and RAPD loci. The average microsatellite values for *P* and *He* were 0.90 (range: 0.45–1.00) and 0.50 (range 0.094–0.736), as compared with allozyme values of 0.200 (range 0.100–0.308) and 0.068 (range 0.026–0.116), and RAPD values of 0.524 (range 0.407–0.627) and 0.188 (range 0.138–0.213), respectively. These results show that microsatellite loci are more polymorphic than allozyme and RAPD loci among wild wheat populations from Israel and Turkey.

Genetic distance

Coefficients of genetic distance *D* were calculated for pair-wise comparisons of the 15 populations (Nei 1972). A matrix of genetic-distance values for the 15 wild wheat populations is presented in Table 4. The genetic distances for all 105 pairs ranged from 0.876 to 3.320 and averaged 1.862 (Table 4). The highest genetic distance (3.320) was obtained between the population of Bet-Oren and the population of Tabigha, while the most-similar populations were Amirim and Jaba with a genetic distance of 0.876. In many cases, the estimates of *D* were geographically independent. They displayed large *D*s, i.e. sharp genetic divergence over very short geographic distances, against low *D*s between geographically distant populations. For example, the genetic distance obtained between the Bat-Shelomo and Bet-Oren populations, located only about 15 km apart, was 2.776, whereas the genetic distance between the Mt. Gilboa population in Israel and the Diyarbakir population in Turkey, separated by 750 km, was only 1.122. Many other such examples occur and are given in Table 4. This suggests that geographic distance alone may not explain interpopulation genetic divergence, which rules out an isolation by distance model (Wright 1943).

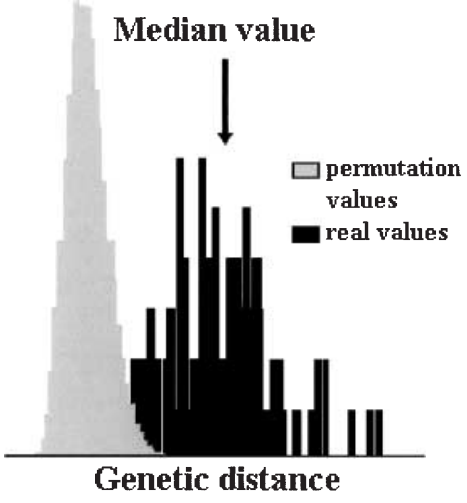

Fig. 3 Distribution of pair-wise genetic distances for real and re-shuffled data of 15 wild emmer wheat populations from Israel and Turkey. The distribution of genetic distance data obtained by the permutation test (10000 replications) was plotted on the same scale together with the real genetic distance values obtained for 15 wild emmer wheat populations from Israel and Turkey

The significance of the revealed between-population differentiation was evaluated by means of a permutation test. In each run of the test, the 135 genotypes were randomly subdivided into 15 populations while maintaining the sizes of the populations the same as was in the real data. For each such subdivision, the pairwise distances were re-calculated exactly in the same way as it was in the real (not re-shuffled) data. Such runs have been repeated 10,000 times. It appeared that only 10% of distances from the re-shuffled data exceeded the minimum pair-wise distance of the real data, and none of the distances for re-shuffled data reached the median distance for real data (Fig. 3). Thus, the pair-wise distances exceeding the median are significant (i.e. cannot be ascribed to a sampling effect) at the level of at least $p<10^{-4}$.

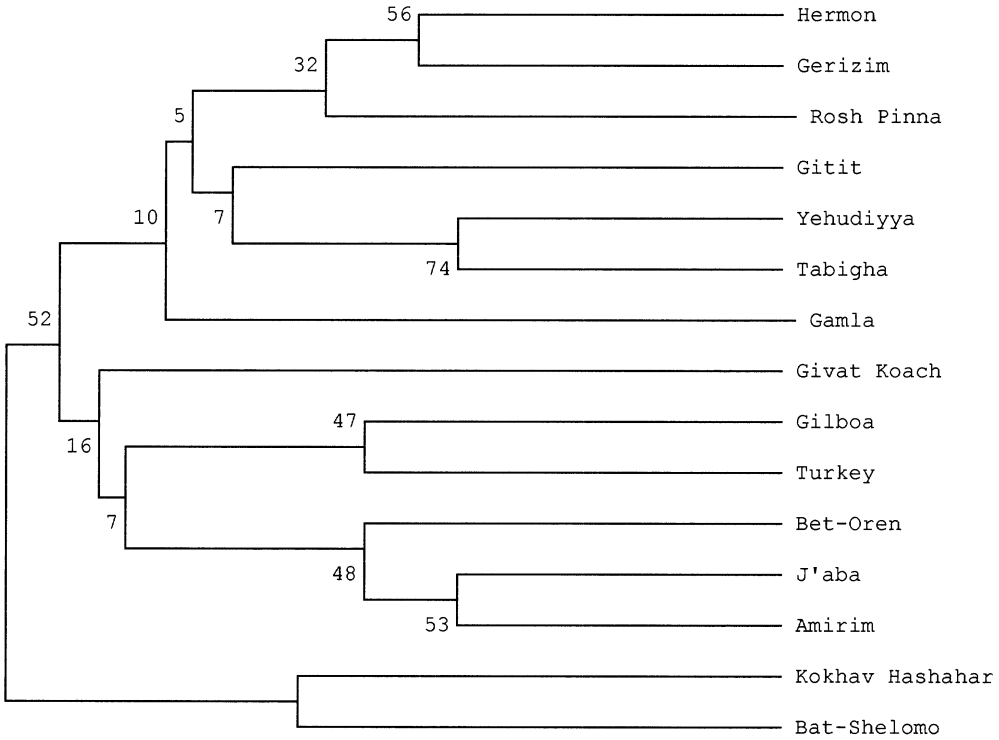

Fig. 4 Dendrogram of 15 wild emmer wheat populations from Israel and Turkey based on the genetic distance calculated from data of 20 wheat microsatellites, using UPGMA as the clustering method. Percentage bootstrap values calculated by re-sampling loci over 1000 replications are displayed above the branches

A consensus tree of 1000 bootstraps of genetic distances was constructed using the UPGMA method (Fig. 4). The obtained dendrogram discriminates all the wild wheat populations tested. Two major and several minor groups can be distinguished (Fig. 4). The obtained dendrogram shows that the pattern of clustering of most of the *T. dicoccoides* populations is related to the eco-geographic distribution of the wild wheat populations. The two major groups, obtained 52% of the time in bootstrap analysis, differentiate the northern populations located near the Sea of Galilee (Tabigha and Rosh-Pinna) and the Golan Heights (Gamla, Yehudiyya and Mt. Hermon) from the southern populations located in Judea, Samaria, the Carmel mountains, and the costal plains (Jaba, Kokhav Hashahar, Gilboa, Bat-Shelomo, Beit-Oren, Givat Koach). However, some of the clustering is between populations that are located in different geographic regions but share similar ecological conditions. For example, the Mt. Hermon and the Mt. Gerizim populations clustered together in 56% of the estimated trees, although they are located 120-km apart. This clustering may be explained by similarity in the ecological conditions: both are located on high mountain tops with high precipitation, an altitude of 1300 m and 800 m, and rainfall of 1400 and 800 mm, for Mt. Hermon and Mt. Gerizim respectively. Furthermore, these two populations are both located on terra rossa soil.

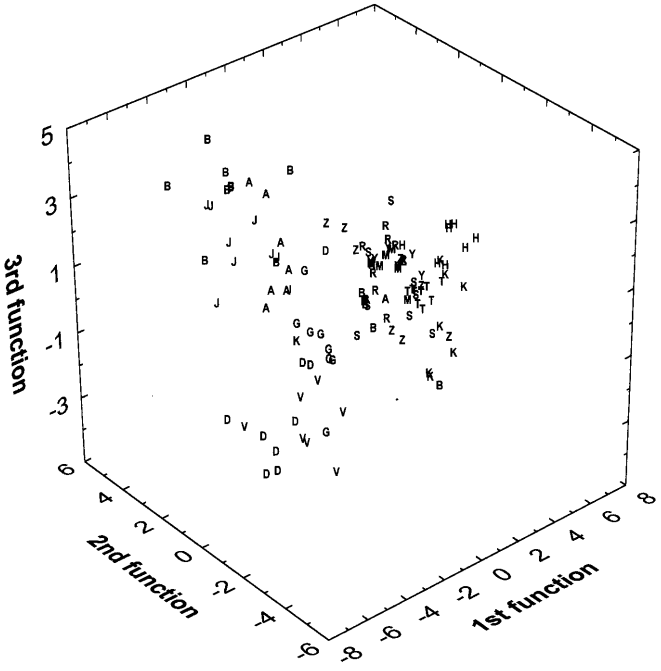

Fig. 5 Plot of canonical discriminant functions 1, 2 and 3 based on ten polymorphic microsatellite loci of 15 populations of wild emmer wheat, *T. dicoccoides*, from Israel and Turkey. Symbols: *H*=Hermon; *Y*=Yehudiyya; *M*=Gamla; *R*=Rosh Pinna; *T*=Tabigha; *B*=Mt. Gilboa; *Z*=Mt. Gerizim; *G*=Gitit; *K*=Kokhav Hashahar; *J*=J'aba; *A*=Amirim; *B*=Bet-Oren; *S*=Bat-Shelomo; *V*=Givat-Koach; *D*=Diyarbakir

Discriminant analysis

On the basis of allele frequencies, the discriminant analysis succeeded to differentiate significantly, almost all the 15 populations. The summary of results is presented in Tables 5 and 6, and Fig. 5. The following ten loci were chosen as the best differentiating factors: GWM251, GWM60, GWM120, GWM169, GWM95, GWM186, GWM18, GWM99, GWM218 and GWM389

Table 5 Summary table obtained by stepwise discriminant analysis of allele frequencies between 15 populations of wild emmer wheat, *T. dicoccoides* in Israel and Turkey, based on ten chosen microsatellite loci

Locus		Number of variables in	F to enter-remove	Prob>*F*	Wilk's lambda
1	GWM251	1	32.314	0.0000005	0.2096
2	GWM60	2	15.860	0.0000005	0.0731
3	GWM120	3	13.730	0.0000005	0.0278
4	GWM169	4	13.445	0.0000005	0.0106
5	GWM95	5	10.408	0.0000005	0.0047
6	GWM186	6	9.049	0.0000005	0.0022
7	GWM18	7	7.498	0.0000005	0.0011
8	GWM99	8	5.957	0.0000005	0.0006
9	GWM218	9	5.778	0.0000005	0.0003
10	GWM389	10	5.207	0.0000005	0.0002

Table 6 Classification of 135 individual genotypes from 15 populations of wild emmer wheat, *T. dicoccoides*, from Israel and Turkey into their respective populations using discriminant analysis based on ten microsatellite loci

No.[a]	Population[b]	1	7	8	9	11	16	17	18	19	23	24	28	30	33	36	Correct classification (%)
1	**Mt. Hermon**	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	89
7	**Yehudiyya**	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	100
8	**Gamla**	0	0	8	1	0	0	0	0	0	0	0	0	0	0	0	89
9	**Rosh-Pinna**	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	100
11	**Tabigha**	0	1	0	0	8	0	0	0	0	0	0	0	0	0	0	89
16	**Mt. Gilboa**	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	100
17	**Mt. Gerizim**	0	0	0	0	2	0	7	0	0	0	0	0	0	0	0	78
18	**Gitit**	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	100
19	**Kokhav Hashahar**	0	0	0	0	0	0	0	1	8	0	0	0	0	0	0	89
23	**J'aba**	0	0	0	0	0	0	0	0	0	6	1	2	0	0	0	67
24	**Amirim**	0	0	0	0	0	0	1	0	0	0	6	2	0	0	0	67
28	**Bet-Oren**	0	0	0	0	0	0	0	0	0	2	0	7	0	0	0	78
30	**Bat-Shelomo**	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	100
33	**Givat Koach**	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	100
36	**Diyarbakir,**	0	0	0	0	0	0	1	0	0	0	0	0	0	1	7	78
Total		8	11	8	10	10	9	9	10	8	8	7	11	9	10	7	88

[a] Population numbers are according to Nevo and Beiles (1989)
[b] Sample size of nine accessions per population

(Table 5). The correct classification of individual genotypes into their respective populations according to their geographic distribution ranged between 67% and 100%, with an average of 88%. The first, second, and third canonical discriminant functions are illustrated graphically in Fig. 5. These results indicate that microsatellite markers can be used to differentiate *T. dicoccoides* genotypes and classify them according to their respective populations, based on multilocus analysis of non-coding genomic regions. Figure 5 shows that in most cases the genotypes of each population are clustered together. In addition, most of the central populations from eastern Galilee (Rosh-Pinna and Tabigha) and the Golan Heights (Gamla, Yehudiyya, and Mt. Hermon) are clustered together, with extensive overlapping between them. By contrast, the peripheral small populations from Judea (J'aba and Gitit), the coastal plain (Givat Koach) and Mt. Carmel (Bet-Oren) in Israel, and Diyarbakir in Turkey, show a different pattern of clustering. The peripheral populations show clear separation from each other, with almost no overlapping between them, and they can be easily distinguished from the major cluster of the central populations. Most of these are isolated marginal populations that can also be distinguished by their unique ecogeographical conditions.

Multiple regression analysis of environmental variables and microsatellite polymorphisms

The best predictors of *P*, *He*, Shannon's information index, the allele number per locus, and representative allele frequencies (alleles that were present at least in four populations) at the 20 polymorphic GWM loci in the 15 wild wheat populations were tested by stepwise multiple regression (MR) analysis. These characters were employed as dependent variables, and geographic, climatic and edaphic factors served as independent variables. The following environmental variables were included in the analysis. *Geographical*: longitude (Ln), latitude (Lt), altitude (Al), and *climatic* means: *temperature*, annual (Tm), January (Tj), August (Ta), seasonal

Table 7 Coefficient of multiple regressions (R^2) of genetic indices, allele number per locus and allele frequencies as the dependent variables and environmental variables in 15 populations of wild emmer wheat, *T. dicoccoides*, in Israel and Turkey as independent variables. Symbols of variables are explained in the text and in Table 1

Genetic indices	**Stepwise model by ecogeographical variables**		
	Step 1	Step 2	Step 3
P	Huan 0.196@		
He	Al 0.199@	Al Ta 0.382*	
Shanon's information index	Al 0.232@	Al Ta 0.389*	Al Ta Rn 0.492*
Allele number/locus			
GWM95	Rn 0.283*		
GWM120	Td 0.242@	Td So 0.481*	Td So Ln 0.579*
GWM154	Al 0.431**	Al Ta 0.607**	Al Ta Rn 0.744**
GWM155	So 0.327*	So Trd 0.502*	So Trd Ta 0.555*
GWM186	Al 0.318*	Al Huan 0.389@	Al Huan Rn 0.512*
GWM234	Al 0.252@	Al Dw 0.506*	Al Dw Rd 0.574*
GWM251	Rn 0.553**	Rn Huan 0.696***	Rn Huan Trd 0.750**
GWM265	Huan 0.188	Huan Rv 0.470*	Huan Rv Ln 0.588*
GWM408	Rv 0.293*	Rv Ln 0.399*	Rv Ln Trd 0.481@
Allele frequency			
GWM18-B	Ta 0.304*		
GWM18-F	Huan 0.301*	Huan So 0.429*	Huan So Rn 0.589*
GWM60-K	Rr 0.171ns	Rr Ln 0.344@	Rr Ln Rn 0.540*
GWM60-L	So 0.465**	SoTj 0.517**	
GWM95-C	Hu14 0.321*		
GWM95-L	So 0.296*	So Rr 0.483*	
GWM99-F	So 0.298*		
GWM99-Q	Huan 0.285*	Huan Rv 0.406*	
GWM120-K	Rn 0.322*		
GWM120-M	Huan 0.273*	Huan Td 0.448*	
GWM154-F	Ta 0.208@	Ta Trd 0.525*	Ta Trd Al 0.624**
GWM154-G	Huan 0.327*	Huan So 0.475*	Huan So Rn 0.535*
GWM155-C	Trd 0.441**	Trd Ta 0.541**	
GWM155-K	Rn 0.369*	Rn Ta 0.648**	Rn Ta Rd 0.708**
GWM186-O	So 0.300*	So Hu14 0.481*	
GWM218-B	Trd 0.257*		
GWM218-J	So0.341*	So Tdd 0.538**	So Tdd Rv 0.583*
GWM219-D	So 0.468**		
GWM219-E	Tm 0.279*	Tm Huan 0.353@	Tm Huan Tj 0.562@
GWM219-G	Trd 0.292*	Trd Rd 0.522*	Trd Rd Ev 0.646**
GWM234-D	Al 0.344*	Al Rn 0.410*	
GWM234-F	So 0.296*	So Hu14 0.504*	So Hu14 Ta 0.690**
GWM251-B	Ta 0.143ns	TaRn 0.424*	
GWM251-K	So 0.283*		
GWM265-C	Hu14 0.205@	Hu14 Lt 0.579**	Hu14 Lt Ev 0.651**
GWM340-P	Trd 0.280*		
GWM389-Q	Al 0.368*	Al Tm 0.442*	Al Tm Ta 0.557*
GWM400-D	Rd 0.258*	Rd Tm 0.471*	Rd Tm Trd 0.518*
GWM400-F	Ta 0.126ns	Ta Rn 0.426*	
GWM400-H	So 0.104ns	So Rn 0.248ns	So Rn Rd 0.642**
GWM400-J	Huan 0.279*	Huan Ev 0.397*	
GWM408-P	Ta 0.118ns	Ta Rd 0.289ns	Ta Rd Rd 0.797*
GWM577-K	Al 0.240*		

Level of significance: ***=p<0.001; **=p<0.01; *=p<0.05; @=p<0.10; ns=p>0.10

temperature difference (Td), daily temperature difference (Tdd), number of tropical days (Trd), evaporation (Ev); *moisture*, annual rainfall (Rn), number of rainy days (Rd), number of dewy nights in summer (Dw), annual humidity (Huan), humidity at 14:00 (Hu14), inter-annual rainfall variation (Rv), coefficient of variation in rainfall (Rr); and three *edaphic* dummy variables, one per each of the soil types: basalt (Ba), rendzina (Ren) and terra rossa (Tr). The test involved the 15 wild wheat populations. The results are given in Table 7. Altitude, temperature, and water-availability factors, singly or in combination, explained a significant proportion of the diversity in the polymorphism of microsatellites. The best two variable-predictors of *He* and Shannon's information index, explaining significantly 0.38–0.4 of their variance, were Al and Ta (altitude and mean temperature in August). A three-variable combination involving both geographic (altitude) and climatic (mean temperature in August and mean annual rainfall) factors, AlTaRn, accounted significantly (p=0.05) for 0.49 of the variance in *P* and Shannon's information index.

Based on the number of microsatellite alleles per locus, microsatellite loci can be classified into several categories in terms of their prime ecogeographical predictors: (a) water factors (Rn, Hu, Rv): GWM95, GWM251, GWM265, GWM408; (b) soil type (So):

GWM155, GWM; (c) temperature (Td): GWM120; (d) geographic factors (Al): GWM154, GWM186, GWM234; (e) water+temperature (Rn, Hu, Trd): GWM251; (f) soil+temperature (So, Trd, Td, Ta): WGM120, GWM155; (g) geographic factors+temperature (Al, Ta): GWM154; (h) geographic factors + water (Al, Ln, Dw, Rn, Hu, Rv, Rd): GWM234, GWM186, GWM408, GWM265. Microsatellite alleles can be similarly classified on the basis of allele frequencies, as can be seen in the results presented in Table 7.

Discussion

The main objective of the present study was to assess mostly non-coding microsatellite diversity among wild emmer wheat populations originating mainly from Israel, to test for relationships with ecological parameters, and compare the pattern with coding allozymes. The Gatersleben wheat microsatellites (GWM) were used to detect DNA polymorphism among 15 populations of *T. dicoccoides*, which included 14 populations from Israel and one from Turkey.

Wheat microsatellites

The utility of microsatellite markers in the study of genetic diversity within and between wild and cultivated wheat germ plasm was already demonstrated in our previous study (Fahima et al. 1998). Regions flanking microsatellite loci are often conserved between closely related species (Moore et al. 1991), allowing the use of primers to amplify loci in closely related species. Although the *T. dicoccoides* accessions used in this study originated from a wide range of habitats, and show a high level of polymorphism, only a small proportion of marker-accession combinations failed to amplify a product in *T. dicoccoides*. These missing amplification products are most likely due to sequence alterations, such as point mutations, deletions or inversions, within the priming sites, as reported by Devos et al. (1995). Although the GWM markers were derived from hexaploid wheat (AABBDD), *Triticum aestivum* (Röder et al. 1995, 1998), they were successfully used to study genetic diversity in several cultivated and wild *Triticum* species, including tetraploid wheats (AABB), such as *Triticum durum*, *Triticum aethiopicum* (Plaschke et al. 1995) and *Triticum dicoccoides* (Fahima et al. 1998; Li et al. 2000a,b,c), and in diploid *Triticum* species such as *Aegilops tauchii* (genome DD: Pestsova et al. 2000).

Genetic diversity

In the present study, using 20 GWM, 364 alleles were revealed among 135 wild wheat genotypes, an average of 18 alleles per GWM. In a previous study, using 23 GWM markers, Plaschke et al. (1995) revealed a total of 142 alleles, among 40 cultivated wheat lines, an average of 6.2 alleles per GWM. The present results demonstrate the high diversity in microsatellite sequences among *T. dicoccoides* accessions compared with the cultivated germplasm, as was earlier demonstrated by RAPD (Fahima et al., 1999) and allozyme markers (Nevo and Beiles 1989) markers. Clearly, genetic diversity was drastically eroded during the domestication process in both coding and non-coding genomic regions, as was the case in other major cereal crops (Nevo 1983, 1989, 1995, 2001).

The dendrogram presented in this study (Fig. 4) clearly demonstrates the ability of microsatellites developed on the basis of *T. aestivum* sequences to detect a large amount of genetic diversity in wild emmer wheat and to identify intergroup differences. All the *T. dicoccoides* populations of this collection were distinguishable by the GWM markers used, even within closely related populations originating in close geographic locations. Our results demonstrate that the DNA polymorphism of the wild wheat correlated with the ecogeographic distribution of the accessions. In addition, the Israeli collection studied here exhibited high interpopulation and interregional polymorphism. These observations are consistent with previous results obtained with isozymes and different DNA markers for different collections of wild emmer wheat covering a much wider geographic range (Nevo et al. 1982; Nevo and Beiles 1989; Nevo 1983, 1989, 1995). Remarkably, however, the present results strongly suggest that the non-coding wheat genome is subject to strong natural selection, as is the case for the coding genome regions harboring the genes (see later).

Genetic structure of wild emmer wheat populations

Wild emmer wheat grows in lush and extensive stands in the catchment area of the upper Jordan Valley, in the eastern upper Galilee Mountains and the Golan Heights in Israel. However, elsewhere in the Fertile Crescent, populations of wild emmer are semi-isolated and isolated, and largely display a patchy structure (Nevo et al. 1982; Nevo and Beiles 1989). Microsatellite analysis was found to be highly effective in distinguishing genotypes and populations of *T. dicoccoides* originating from these diverse ecogeographic sites in Israel and Turkey, as was demonstrated by the high level of polymorphic loci, genic diversity, and Shannon's information index (Table 3), by high genetic distance values between populations (Table 4, Fig. 4), and by discriminant analysis (Table 6, Fig. 5). In this study 88% of the 135 genotypes were correctly classified into sites of origin by discriminant analysis on the basis of microsatellite genotyping (Table 6). The genotypes chosen for this study spanned most of the ecological range of emmer wheat in Israel. Central populations (Yehudiyya, Gamla, Rosh-Pinna and Tabigha) were collected in warm, humid environments

on the Golan Plateau and near the Sea of Galilee. Marginal steppic populations (Mt. Hermon, Mt. Gilboa, Mt. Gerizim, Gitit, Kokhav Hashahar and J'aba) were collected across a wide geographic area on the northern, eastern, and southern borders of wild emmer distribution, involving hot, cold and xeric peripheries, while marginal mesic (Mediterranean) populations (Amirim, Bet-Oren, Bat-Shlomo and Givat Koach) were collected from the western border of wild emmer distribution (Nevo et al. 1982; Nevo and Beiles 1989). The dendrogram presented in Fig. 4 and the discriminant analysis in Fig. 5 show a clustering together of the central populations and a clear separation of most of the isolated marginal populations (e.g. Mt. Hermon, Givat Koach, Gitit, Bet-Oren and Turkey) from the other populations. These results demonstrate the influence of the unique ecogeographic conditions on the genetic structure of these populations.

Correlation with environmental variables

Wild emmer wheat is distributed over the Fertile Crescent, including Israel, Syria, Jordan, Turkey and Iran. These natural populations are highly polymorphic in morphological characters, as well as in various economically important traits such as disease resistance, grain quality, photosynthetic yield, salt tolerance, herbicide resistance, etc. (for reviews see Nevo 1983, 1989, 1995, 2001). Although major collection areas such as Mt. Hermon, Rosh Pinna, Gamla, Bat-Shelomo and Tabigha are at similar longtitude and latitude, they differ significantly in altitude. These locations, for example, are respectively at 1300, 700, 200, 75 and 0 m above sea level (Table 1). Along with these features, several other environmental factors differ for these locations, such as abiotic climatic conditions, water availability, soil type, and biotic factors such as parasites, pathogens and competitors (Nevo and Beiles 1989; Nevo et al. 1982). Our results show high levels of diversity of microsatellite markers in these wild wheat populations. These polymorphic microsatellite markers showed significant correlates with climatic factors (Table 7) and may be useful in a predictive sense for sampling strategies. Altitude, temperature, and water-availability factors, singly or in combination, explained a significant proportion of the diversity in the poly-morphism of microsatellites. The association of altitude with microsatellite diversity could be explained by the sharp gradient of climatic conditions down the mountain slopes, with increasing temperatures and water availability downslope towards valleys between mountain ridges. Note that the list of climatic factors included in our analysis does not represent all the possible components involved in determination of the real climate, nor does it contain the biotic factors. Thus, we conclude that altitude involves climatic components, but certainly also a host of changing biotic factors.

Genetic diversity detected at microsatellite versus allozyme and RAPD loci

Microsatellite markers have proved to be useful for population genetic analysis in wheat (Plaschke et al. 1995; Fahima et al. 1998; Li et al. 2000a,b,c; Pestsova et al. 2000). In this study we compared genetic diversity at microsatellite loci with the previously studied allozyme (Nevo and Beiles 1989) and RAPD loci (Fahima et al. 1999) in wild emmer wheat populations. Our results show complete agreement among microsatellite, allozyme, and RAPD genetic-diversity profiles. However, microsatellites yielded much higher values of diversities than allozymes and RAPDs. Allozymes have provided useful insights into the genetic structure of wild emmer wheat (Nevo and Beiles al. 1989), but the number of scorable loci is limited and diversity is detected only at coding loci. The RAPD technique (Williams et al. 1990) is a powerful tool for obtaining a large number of anonymous loci of both coding and non-coding sequences, although it is commonly considered that most RAPD loci are non-coding sequences (Williams et al. 1990).

The microsatellite loci studied here were dinucleotide repeats that represent non-coding sequences; however, some of them may reside within untranslated regions of gene sequences (5′ leader, 3′ tailer or intron sequences) or non-transcribed gene sequences (e.g. promoter or terminator regions). Many such examples were found in many species (Kashi et al. 1997; Kashi and Soller 1999). The results showed a higher diversity of microsatellite and RAPD loci as compared with allozyme loci. This may be partially explained by the conserved nature of coding sequences primarily of the housekeeping genes sampled by allozymes versus non-coding sequences sampled by microsatellites and RAPDs. However, considerable evidence exists to indicate that microsatellites in non-coding regions are also of functional importance (reviewed by Kashi et al. 1997; Kashi and Soller 1999). The parallelism of microsatellite, RAPD, and allozyme patterns indicates that similar primarily deterministic (selection) evolutionary forces are involved in shaping the genomic structure of all sampled loci, in coding as well as non-coding genomic regions. This may suggest that the non-coding genomic regions are far from representing 'junk DNA.' On the contrary, they may represent regulatory elements whose exact function awaits critical testing.

Genetic distance versus geographical distance

We tested the relationship between microsatellite genetic distance and geographical distance, and often found that the estimates of genetic distance *D* were geographically independent. They displayed large *D*s, i.e. sharp genetic divergence over very short geographic distances, against small *D*s between geographically distant wild emmer populations. For example, the genetic distance obtained between the Bat-Shelomo population and the Bet-Oren

population, located only about 15 km apart, was 2.4-times higher than the genetic distance between the Mt. Gilboa population in Israel and the Diyarbakir population in Turkey, separated by 750 km (50-times the distance between Bat-Shelomo and Bet-Oren). Many other such examples occurred (Table 4). The absence of a significant relationship between geographic separation and genetic distance *D* attests to a sharp local differentiation rather than a gradual change in allele frequencies across the range of *T. dicoccoides* in Israel. This was previously found for allozymes and RAPDs (Nevo et al. 1982; Nevo and Beiles 1989; Fahima et al. 1999). Population divergence does not seem to follow the simple isolation by distance model of Wright (1943). Quite often a greater genetic difference is easier to find between close populations than between populations that are far apart. This was clearly demonstrated by local short microscale transacts of different soil types at Tabigha and the micro-difference of sun-shade differentiation at Yehudiyya (Nevo et al. 1988a,b; Li et al. 2000a,b). This confirms the island-population genetic model of wild emmer wheat (Nevo and Beiles 1989). Thus, the genetic structure of wild emmer wheat populations in Israel is mosaic. This patchy genetic distribution appears to reflect the underlying ecological heterogeneity at both micro- and macro-scales (Golenberg and Nevo 1987; Nevo et al. 1988a,b; Nevo and Beiles 1989; Fahima et al. 1999; Li et al. 2000a,b,c). The high polymorphism and genetic diversity found within and between populations could be explained by spatiotemporal selection. Moreover, the micro-environmental variation, coupled with limited migration of *T. dicoccoides*, can explain the within-population diversity.

The adaptive nature of microsatellite polymorphisms in wild emmer

Many authors generally consider microsatellites to be neutral DNA markers (e.g. Nauta and Weissing 1996; Awadalla and Ritland 1997; Schlötterer and Wiehe 1999); sometimes they are even considered “junk DNA” (Nowak 1994). Yet microsatellite functional significance has been proven by various critical experiments in many biological phenomena, such as in gene transcription, gene translation, chromatin organization, genome size, recombination, DNA replication, DNA repair, cell cycle, etc. (reviewed by Kashi et al. 1997; Kashi and Soller 1999). Unlike random point mutations, which can cause unpredictable and sometimes disastrous qualitative alterations in gene function, the frequent addition or subtraction of one motif repeat within a gene should commonly cause only a small quantitative adjustment in gene transcription activity (King et al. 1997). This should often result in a correspondingly small increase or decrease in some quantitative aspect of phenotype. Widespread repeat-number polymorphism in functional microsatellites should therefore yield similarly widespread quantitative variation in numerous phenotypic traits.

With their high rate of reversible mutation, their ability to influence gene activity, their ubiquitous distribution, their frequent association with regulatory loci, their site-specific mutation rates, and the low probability that their mutations will be deleterious, microsatellites may facilitate rapid and efficient evolutionary adaptation (King et al. 1997). Therefore, some authors suggest that microsatellites can provide an abundant source of quantitative genetic variation that might be important for Darwinian selection (Kashi et al. 1997) and for artificial selection in domesticated stocks (Kashi et al. 1990, 1997; King 1994; Gerber et al. 1994; Sutherland and Richards 1995). Furthermore, the combination of high mutation rate and regulatory functions raises the possibility that microsatellites are a major source of eukaryotic genetic variation (Kashi and Soller 1999) and a substrate for evolutionary changes (Kashi et al. 1997; King et al. 1997; Kashi and Soller 1999). The evidence presented in this study supports the hypothesis that microsatellite polymorphisms are at least partly adaptive and are determined by natural diversifying selection.

The evidence includes: (1) the association of microsatellite loci with climatic and soil factors; genetic divergence appears largely to match the ecological heterogeneous background, and (2) genetic divergence is not associated with geographic distance, but with local ecological conditions of soil and climate. Our overall results indicate that genetic diversity among localities displays weak and non-significant correlations with geography and stronger ones with ecology. These results are in accordance with those found earlier using allozyme and RAPD markers (Nevo and Beiles 1989; Nevo et al 1982; Fahima et al. 1999). We also found evidence for strong selection on microsatellites in small microsite studies of *T. dicoccoides* in northern Israel, at Ammiad, Tabigha and Yehudiyya (Li et al. 2000a,b,c).

The wild gene-pool of emmer wheat

The microsatellite analysis used in the present work corroborates the evidence of genetic diversity and divergence found in natural populations of *T. dicoccoides* across Israel (Nevo et al. 1982; Nevo and Beiles 1989; Fahima et al. 1999). The populations of *T. dicoccoides* showed great diversity, both between and within populations, emphasizing the importance of this wild wheat-relative in future breeding programs and confirming the proposal that it may be an important source for wheat improvement (Nevo 1983, 1989, 1995, 2001). This idea was first suggested by Aaronsohn (1913), and later elaborated by many authors. Multidisciplinary studies of wild wheat, in Israel, conducted at the Institute of Evolution (reviewed in Nevo 1983, 1989, 1995, 2001) and elsewhere (e.g. Feldman 1979; Grama et al. 1983; Joppa and Cantrell 1990), indicate that wild wheat harbors rich genetic resources for wheat improvement, not only regionally but also locally. Sampling strategies should be designed accordingly, considering *macro*- and *micro*-ecological divergence.

The obtained results suggest that microsatellite markers are useful for the estimation of genetic diversity in wild germplasm of *T. dicoccoides*. These results will be useful in future sampling strategies and the identification of suitable parental lines for the mapping studies aimed to tag agronomically important traits in wheat derived from *T. dicoccoides*. This approach proved useful for selecting suitable parents for mapping of stripe rust resistance genes derived from *T. dicoccoides* based on microsatellite analysis of highly resistant wild wheat lines (Fahima et al. 1998; Peng et al. 1999, 2000a). Further work is now in progress to test the adaptive evolutionary significance of microsatellites in natural populations of wild emmer wheat, both at *macro*- and *micro*-geographic scales. Our current results, showing significant correlations between microsatellites and ecological diversities, suggest that the former are subjected to natural selection, and may serve not only as genetic markers but also as adaptive multilocus genomic probes.

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References

Aaronsohn A (1913) The discovery of wild wheat. City Club Bull Chicago 6:167–173

Amiran DHK, Elster J, Gilead M, Rosenman N, Kadmon N, Paran U (1970) Atlas of Israel. Surveys of Israel Ministry of Labor. Elsevier, Jerusalem and Amsterdam

Awadalla P, Ritland K (1997) Microsatellite variation and evolution in the *Mimulus guttatus* species complex with contrasting mating system. Mol Biol Evol 14:1023–1034

Caudrado A, Schwarzacher T (1998) The chromosomal organization of simple sequence repeats in wheat and rye genomes. Chromosoma 107**:**587–594

Chagué V, Fahima T, Dahan A, Sun GL, Korol AB, Ronin YI, Grama A, Röder MS, Nevo E (1999) Isolation of microsatellite and RAPD markers flanking the *Yr15* gene of wheat using NILs and bulked segregant analysis. Genome 42:1050–1056

Devos, KM, Bryan GJ, Collins AJ, Stephenson P, Gale MD (1995) Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90:247–252

Fahima T, Röder MS, Grama A, Nevo E (1998) Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96:187–195

Fahima T, Sun GL, Beharav A, Krugman T, Beiles A, Nevo E (1999) RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theor Appl Genet 98: 434–447

Feldman M (1979) Genetic resources of wild wheats and their use in breeding. Monogr Genet Agrar 4:9–26

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gerber HP, Seipel K, Georgiev O, Hoefferer M, Hug M, Rusconi S, Schaffner W (1994) Transcriptional activation modulated by homopolymeric glutamine and proline stretches. Science 263:808–811

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

Grama A, Gerechter-Amitai ZK, Blum A (1983) Wild emmer as a donor of genes for resistance to stripe rust and for high protein content. In: Sakamoto S (ed) Proc 6th Int Wheat Genetics Symp, Kyoto University, Kyoto, Japan, pp 178–192

Harlan JR, Zohary D (1966) Distribution of wild emmer wheat and barley. Science 153:1074–1080

Joppa LR, Cantrell RG (1990) Chromosomal location of genes for grain protein content of wild tetraploid wheat. Crop Sci 30:1059–1064

Kashi Y, Soller M (1999) Functional roles of microsatellites and minisatellites. In: Goldstein DB, Schlötterer C (eds) Microsatellites: evolution and application. Oxford University Press, Oxford, pp 10–23

Kashi Y, Tikochinsky Y, Iraki F, Beckmann JS, Gruenbaum Y, Soller M (1990) Large DNA fragments containing poly(TG) are highly polymorphic in a variety of vertebrates. Nucleic Acids Res 18:1129–1132

Kashi Y, King D, Soller M (1997) Simple sequence repeats as a source of quantitative genetic variation. Trends Genet 13:74–78

King DG (1994) Triple repeat DNA as a highly mutable regulatory system. Science 263:595–596

King DG, Soller M (1999) Variation and fidelity: the evolution of simple sequence repeats as functional elements in adjustable genes. In: Wasser SP (ed) Evolutionary theory and processes: modern perspectives. Papers in honor of Eviatar Nevo. Kluwer, The Netherlands, pp 65–82

King D, Soller M, Kashi Y (1997) Evolutionary tuning knobs. Endeavour 21:36–40

Korzun V, Börner A, Worland AJ, Law CN, Röder MS (1997) Application of microsatellite markers to distinguish intervarietal chromosome substitution lines of wheat (*Triticum aestivum* L.). Euphytica 95:149–155

Korzun V, Röder MS, Ganal MW, Worland AJ, Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 96:1104–109

Li YC, Fahima T, Peng JH, Röder MS, Kirzhner V, Beiles A, Korol AB, Nevo E (2000a) Edaphic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. Theor Appl Genet 101:1029–1038

Li YC, Fahima T, Korol AB, Peng JH, Röder MS, Kirzhner V, Beiles A, Nevo E (2000b) Microsatellite diversity correlated with ecological-edaphic and genetic factors in three microsites of wild emmer wheat in north Israel. Mol Biol Evol 17:851–862

Li YC, Röder MS, Fahima T, Kirzhner V, Beiles A, Korol AB, Nevo E (2000c) Natural selection causing microsatellite divergence in wild emmer wheat at the ecologically variable microsite at Ammiad, Israel. Theor Appl Genet 100: 985–999

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Moore SS, Sargeant LL, King TJ, Mattick JS, Georges M, Hetzel JS (1991) The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10: 654–660

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143**:**1021–1032

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

Nei M (1978) Estimation of average heterozygosity and genetic distance from small number of individuals. Genetics 89:583–590

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

Nevo E (1989) Genetic resources of wild emmer wheat revisited: genetic evolution, conservation and utilization. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, Cambridge, England, pp 121–126

Nevo E (1995) Genetic resources of wild emmer, *Triticum dicoccoides* for wheat improvement: news and views. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp, China Agric Sci Press, Beijing, China, pp 79–87

Nevo E (2001) Genetic resources of wild emmer, *Triticum dicoccoides*, for wheat improvement: in the third millenium. Israel J Plant Sci (in press)

Nevo E, Beiles A (1989) Genetic diversity of wild emmer wheat in Israel and Turkey. Theor Appl Genet 77:421–455

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

Nevo E, Beiles A, Krugman T (1988a) Natural selection of allozyme polymorphisms: a microgeographic climatic differentiations in wild emmer wheat (*Triticum dicoccoides*). Theor Appl Genet 75:529–538

Nevo E, Beiles A, Krugman T (1988b) Natural selection of allozyme polymorphisms: a microgeographical differentiation by edaphic, topographical, and temporal factors in wild emmer wheat (*Triticum dicoccoides*). Theor Appl Genet 76:737–752

Nowak R (1994) Mining treasures from 'junk DNA.' Science 263:608–610

Peng JH, Fahima T, Röder MS, Li YC, Dahan A, Grama A, Ronin YI, Korol AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides,* and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98: 862–872

Peng JH, Fahima T, Röder MS, Li YC, Grama A, Nevo E (2000a) Microsatellite high-density mapping of the stripe rust resistance gene *YrH52* region on chromosome 1B and evaluation of its marker-assisted selection in the F_2 generation in wild emmer wheat. New Phytol 146:141–154

Peng JH, Korol AB, Fahima T, Röder MS, Ronin YI, Li YC, Nevo E (2000b) Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Peng JH, Fahima T, Röder MS, Huang QY, Dahan A, Li YC, Grama A, Nevo E (2000c) High-density molecular map of chromosome region harboring the *YrH52* gene and its preliminary discrimination from *Yr15* in wild emmer wheat, *Triticum dicoccoides*. Genetica 109:199–210

Pestsova E, Korzun V, Goncharov NP, Hammer K, Ganal MW, Röder MS (2000) Microsatellite analysis of *Aegilops tauschii* germplasm. Theor Appl Genet 101:100–106

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Plaschke J, Börner A, Wendehake K, Ganal MW, Röder MS (1996) The use of wheat aneuploids for the chromosomal assignment of microsatellite loci. Euphytica 89:33–40

Röder MS, Plaschke J, Konig SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schlötterer C, Wiehe T (1999) Microsatellites, a neutral marker to infer selective sweeps. In: Goldstein AB, Schlötterer C (eds) Microsatellites: evolution and applications. Oxford University Press, Oxford, pp 238–247

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana

Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco

Statsoft (1996) STATISTICA for Windows [computer program manual]. Tulsa, Oklahoma: Statsoft, Inc, 2300 East 14th Street, Tulsa, OK 74104, USA

Sutherland GR, Richards RI (1995) Simple tandem DNA repeats and human genetic disease. Proc Natl Acad Sci USA 92:3636–3641

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wright S (1943) Isolation by distance. Genetics 28:114–138

Yeh FC, Yang RC, Boyle T, Ye ZH, Mao JX (1977) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada

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