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Spatio-temporal genetic variation in populations of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, as revealed by AFLP analysis

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Abstract Genetic structure of natural populations of wild crop relatives has been the subject of many studies. Yet, most of them focused on the assessment of spatial genetic diversity, while information on long-term variation, affected by yearly changes, has been considered only in few cases. The present study aimed therefore, to estimate the spatio-temporal genetic variation in populations of wild emmer wheat, the progenitor of domesticated wheat, and to assess the contribution of spatial versus temporal factors to the maintenance of genetic variation in a population. Single spikes were collected in the years 1988 and 2002 from plants that grew in the same sampling points, from six different habitats in the Ammiad conservation site, Eastern Galilee, Israel. Seeds were planted in a nursery and DNA was extracted from each plant and analyzed by the AFLP method. Fourteen primer combinations yielded 1,545 bands of which 50.0 and 48.8% were polymorphic in the years 1988 and 2002, respectively. Genetic diversity was much larger within populations than between populations and the temporal genetic diversity was considerably smaller than the spatial one. Nevertheless, population genetic structure may vary to some degree in different years, mainly due to fluctuations in population size because of yearly rainfall variations. This may lead to predominance of different genotypes in different years. Clustering the plants by their genetic distances grouped them according to their habitats, indicating the existence of genotype-environment affinities. The significance of the results in relation to factors affecting the maintenance of polymorphism in natural populations is discussed.

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Introduction

Allelic polymorphism in natural plant populations is maintained *inter alia* by the environment heterogeneity; the higher the spatial or temporal environmental heterogeneity, the larger the genetic polymorphism maintained in the population (Hedrick et al. 1976; Spieth 1979; Li et al. 2001). Yet, while the impact of spatial heterogeneity on the genetic diversity was studied in many plant populations, that of temporal changes was studied relatively little (reviewed in Li et al. 2001). Such information on the spatio-temporal variation within and between plant populations and the relationships of this variation to ecological conditions is imperative for a more comprehensive understanding of evolutionary dynamics. For wild crop relatives this information is also instrumental for adopting appropriate strategies for conservation and utilization of the valuable wild germplasm (Bretting and Widrlechner 1995), including wild wheat (e.g., Horovitz and Feldman 1991; Anikster et al. 1995), which is threatened by urban expansion,

highway construction and industrial developments. Assessment of genetic divergence, both within- and between-populations in space and between generations in time, is crucial in planning an effective in situ conservation. By in situ conservation evolutionary potential of plant species, and their populations are preserved (Horovitz and Feldman 1991; Anikster et al. 1995).

Wild emmer wheat, *Triticum turgidum* L. ssp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell. ($2n = 4x = 28$; genome BBAA), the progenitor of all important domesticated wheat (reviewed in Feldman et al. 1995; Feldman 2001), is genetically very close to domesticated wheat, and its large gene pool contains many valuable alleles that are easily exploitable for wheat improvement (for review see Feldman 1977; Feldman and Millet 1995; Nevo 2001). Wild emmer wheat is an annual, predominantly self-pollinating grass, native to the "Fertile Crescent" region of Southwest Asia, where it thrives in the transition zone between the Mediterranean and the steppe phytogeographic provinces (Harlan and Zohary 1966; Feldman 1977; Kimber and Feldman 1987; Feldman 2001). The species grows in a diversity of primary and secondary habitats and floristic associations and may be found growing from 200 m below to 1,700 m above sea level, primarily on red soils ("terra rossa"), derived from well-drained, hard limestones, and on various basalts (Kimber and Feldman 1987).

An in depth spatio-temporal study of populations of wild emmer wheat was launched in 1984 in a site near the settlement of Ammiad, north of the Sea of Galilee in Northeastern Israel, by the Scientific Board of the Israeli Gene Bank for Agricultural Crops (Horovitz and Feldman 1991; Anikster et al. 1995). This study (Nevo et al. 1991; Felsenburg et al. 1991) focused on the genetic structure of wild emmer populations inhabiting several different habitats at the Ammiad site. The site, situated at the center of the geographical distribution and diversity of wild emmer in Israel, is ecologically heterogeneous and has been classified into several different habitats (Anikster and Noy-Meir 1991; Horovitz and Feldman 1991). During this study (Nevo et al. 1991; Felsenburg et al. 1991), spatial and, to some extent, also temporal variations were assessed. But, there is not yet a clear picture of either the relationship between within- and between-populations genetic variations or the genetic stability over time in the various habitats.

The present study aimed to further characterize spatial and temporal genetic diversity in several of the Ammiad populations in order to test (1) the conclusion of Hamrick and Godt (1990) that in annual, self-pollinating species that do not disperse genes through pollen or seeds to a long distance, most of the genetic variation is between- rather than within-populations, and (2) the assumption of Karlin (1982) that spatial variation is more effective than temporal variation in protecting a polymorphism in a population. To accomplish these, we analyzed plants that were progenies of seeds that were collected in the years 1988 and 2002 from the same sampling points by applying amplified fragment length polymorphism (AFLP) analysis. The AFLP technique has been extensively employed in studies of genetic diversity within and between plant populations and found most appropriate for studying a large number of loci, particularly in populations of self-pollinating plants (Nybom 2004; Bensch and Åkesson 2005). Since it was planned to analyze a large number of loci and since wild emmer wheat is a self-pollinating species, the AFLP technique is most appropriate for estimating spatial and temporal genetic diversity in populations of this important wild relative of wheat.

Materials and methods

Plant material

Seeds originating from 120 single plants of wild emmer wheat, of which 60 were sampled in the year 1988 and the remaining 60 were sampled from the same points in 2002, from six different habitats in Ammiad, Israel, about ten plants in each habitat, were used in this study (Supplementary Materials Table S1). The sampling points were separated by about 5 m from one another. Ecological descriptions of the six habitats and the method of their collection and propagation were described by Anikster and Noy-Meir (1991), who divided the totality of Ammiad site into the following four groups: Valley (containing three habitats), North (containing two habitats), Ridge (containing four habitats), and Karst (containing two habitats). For purposes of the present study, two habitats from each of the groups, North (N1 and N2), Ridge (Re and Rp2), and Karst (K1 and K2), were chosen. The ecological conditions of these habitats are described in Supplement Materials Table S1. The monthly rainfall distribution in Ammiad during four growing seasons, two in the years 1987–1988 and 2001–2002, and two in the previous years 1986–1987 and 2000–2001, are presented in Supplementary Materials Table S2.

Fluorescent based AFLP analysis

Genomic DNA was extracted from leaves of 1 to 1.5 month old plants by the CTAB method as described by Kidwell and Osborn (1992). Amplified fragment length polymorphism (AFLP) analysis was carried out as described by Vos et al. (1995), with minor modifications. The primers and adaptors used in this study are given in Supplementary Materials Table S3. Altogether 14 primer combinations were used (Supplementary Materials Table S4). Selective amplification was done with *Eco*RI and *Mse*I primers having

three additional nucleotides. For fluorescent-based fragment analysis, *Eco*RI primers selective 2 and selective 5 were labeled with TET (4, 7, 2′, 7′-Tetrachloro-6-carboxyl fluorescein) while selective 3 and selective 8 were labeled with HEX (4, 7, 2′, 4′, 5′, 7′-Hexachloro-6-carboxyfluorescein) (Eisenberg Bros. Ltd) fluorescent dye. Electrophoresis of 3 μl selective amplification products from each sample was done at 2,000 V for 1.5 h. The gel was scanned with a Typhoon 9400 Scanner (Ammersham Bioscience) with the appropriate laser, at medium sensitivity, and intensity of the digital image of the gel was adjusted using the Image Quant software (Supplied with Typhoon Scanner).

Data analysis

Wild emmer wheat is a predominantly self-pollinating plant with less than 1% outcrossing (Golenberg 1988; Felsenburg et al. 1991). Consequently, a very low proportion of heterozygosity is expected and the analysis of dominant markers like AFLP becomes less problematic (Lynch and Milligan 1994). Polymorphic bands, exhibiting presence or absence of bands, were scored as alternative alleles. The data thus obtained were analyzed by GDA (Genetic Data Analysis; Lewis and Zaykin 2001) version 1.1 which is a software package for analysis of discrete population genetics data according to Weir (1996). The data were considered as haploid.

Gene diversity (*He*) (Nei 1973), estimating within-population diversity, was computed as the expected heterozygosity based on allele frequencies for each locus and for all loci, polymorphic and non-polymorphic ones. When applied to predominantly self-pollinating plant species where heterozygotes are infrequent, this index should yield rather accurate estimations also for dominant markers (Lynch and Milligan 1994). The significance of the differences between *He* values of the different populations or years was determined by the *t*-test based on the variance of *He* among the loci.

Genetic differentiation between populations is often estimated with the Nei's Coefficient G_{ST} (Nei 1973) for dominantly inherited DNA markers. This coefficient may tell us how genetic variation is partitioned between within- and between-populations; a high G_{ST} value indicates that plants within a population are relatively similar but populations are considerably different. When there are two alleles (1 and 0), this G_{ST} is identical to Wright's F_{ST} (Nei 1973). Thus, *F* statistics was used to estimate the extent of differentiation between groups (populations and sub-populations) (Hartl and Clark 1997). The coancestry coefficient θ (=F_{ST}) was measured for every locus and for all loci in each year. In another analysis, data of the 2 years were considered as two groups each containing the six populations (habitats) where θ–p estimated the differentiation between

years and θ–s the differentiation between populations. Confidence limits for the estimated θ values were obtained by bootstrapping 5,000 times across loci.

Analysis of variance (AMOVA; Excoffier et al. 1992) based on 1,545 AFLP bands in six populations and 2 years was performed, using the software Arlequin ver 2.000. First, each year was analyzed separately as one group with six populations. A combined analysis of all data was carried out too, based on two groups (years) containing the six populations.

Distance matrix was generated based on all bands according to Nei (1978) and was used for building relatedness tree of the populations using UPGMA medthod.

Results

AFLP analysis

The reproducibility of our scoring of the AFLP profiles was checked by analyzing the genotyping error rate in repeated runs of the same extracts from several plants. The mean number of duplicate samples was 5.3 ranging from three to six in the various primer combinations. Since extraction and PCR amplification of DNA from plants collected from the same sampling points in the years 1988 and 2002 were done at the same time, error rate was assessed only in repeated runs of the DNA on different polyacrylamide gels by scoring the obtained AFLP profiles. Altogether the duplicate samples were run on 14 different gels. The percent errors per duplicate sample, defined as the frequency of differences observed between two runs of the same sample (Bonin et al. 2004), varied from 0.84 in the primer combination ES8 × MS1 to 6.95 in ES2 × MS2; the mean percent errors for all the primer combinations were 2.96. Thus, to be on the safe side, a locus was considered polymorphic in this work only if more than 5% of the plants exhibited a change in its banding pattern.

Overall, 120 plants, 60 collected from six different habitats in the year 1988 and 60 collected from the same sampling points in 2002, were analyzed by the AFLP method. Only bands with lengths between 50 and 500 bp were included in the analysis. The 14 primer combinations used in this study (Supplementary Materials Table S4), four *Eco*RI selective primers that were labeled by four different fluorescent dye, and eight non labeled *Mse*I selective primers (Supplementary Materials Table S3), generated 1,545 bands for each plant (about 110 per primer combination). Altogether there were 185,400 (120 plants × 1,545 bands) observations. In the year 1988, 773 (50.0%) of these bands were monomorphic (of which 29.6% were true monomorphic and 20.4% exhibited different banding pattern in 5% or less of the plants) and 772 (50.0%) polymorphic, and in the year 2002,

791 (51.2%) were monomorphic (of which 31.3% were true monomorphic and 19.9% exhibited different banding pattern in 5% or less of the plants) and 754 (48.8%) polymorphic (Supplementary Materials Table S4). It is noteworthy that every plant had a unique banding profile. In the cluster analysis of all plants (data not shown), plants of the same sampling points that were collected in the years 1988 and 2002 were closer to one another than plants from different sampling points of the same habitat which, in turn, were closer to one another than plants of different habitats.

Spatio-temporal genetic diversity

The percentage of polymorphic loci (P) and gene diversity (He) values for each of the six studied populations are given in Table 1. In both years, 1988 and 2002, higher P values were found in the habitats N1, N2 and Rp2. Populations N1, N2, Rp2 and K2 had significantly higher gene diversity values than population Re. On the other hand, gene diversity values did not differ significantly between years (Table 1). In populations N1, N2 and Rp2, the high percentage of polymorphic loci was accompanied by a high value of gene diversity and in population Re lower percentage of polymorphic loci was accompanied by a lower value of gene diversity. In the year 1988 there were more unique alleles per population than in 2002 (data not shown).

The frequency of loci that were monomorphic in all the six populations in 1988, in 2002 and in both years was 50.0, 51.1, and 45.9%, respectively, while that of polymorphic loci was much lower, 16% in 1988, 13% in 2002, and 9.6% in both years.

Between-populations diversity was estimated by the θ values (Table 2). Hartl and Clark (1997) ascribed different levels of differentiation to different θ values. θ values were calculated for each locus and values presented in Table 2

are overall values. Bootstraping of 5,000 cycles gave confidence limits to θ values. The between-populations relative diversity in the year 1988 showed a moderate value of 0.1243 (confidence limit of 0.1121–0.1368) and that of 2002 showed a significantly larger value of 0.1762 (confidence limit of 0.1610–0.1915). The within-populations relative genetic diversity (calculated as 1.0 minus θ) was 0.8757 for the year 1988 and 0.8238 for the year 2002, both much larger than the between-populations relative genetic diversity.

To calculate the between-years relative genetic diversity (the temporal diversity), the habitats were considered as sub-populations of the years (i.e., populations from habitats that were collected in the year 1988 were considered as sub-populations of year 1988 and similarly to year 2002). The value, θ–p, was calculated as an estimate of the between-years relative genetic diversity (Table 2). θ–p was only −0.0371 (confidence limit −0.0404 to −0.0339) while the combined value for between-populations diversity (θ–s) was 0.1192 (confidence limit 0.1083–0.1301). A small negative value of θ is referred as 0 (see Weir 1996, p. 175). Apparently, the differentiation by time is considerably less than that by space.

The results of the analysis of variance are presented in Table 2. It is clear that the percentages of variations are essentially identical to their corresponding θ values (except for θ–s of all the populations that was somewhat lower than the AMOVA between-population relative variation; Table 2), and show that within-populations percentage of variation is much higher than between-populations diversity and that the temporal differentiation is considerably smaller.

The moderately positive relationships between the θ values between populations of the year 1988 with those of 2002 (θ of 88 and θ of 02), that are evident in Supplementary Materials Figure S1, are reflected in a positive ($R = 0.654$) and highly significant ($P < 0.0001$) correlation coefficient, indicating similar trend of spatial diversity among loci in the 2 years.

Genetic divergence

Genetic distance (GD) was calculated from Nei's (1978) formula. GD between plants from the same population in the two studied years varied relatively little, ranging from 0.000 (between K1 88 and K1 02) to 0.011 (between N2 88 and N2 02; Table 3). On the other hand, the GD between populations was much larger ranging from 0.016 (between N1 88 and N2 02) to 0.096 (between N1 02 and K2 02). The higher similarity between samples of the two years within each population is evident in the dendrogram (Supplementary Materials Figure S2). In the dendrogram, populations were clustered in three major groups. Populations

Table 1 Genetic diversity estimates for six wild emmer populations in 1988 and 2002

Population	1988			2002		
	N	P	He	N	P	He
N1	8.6	0.40	0.153a	8.5	0.35	0.141b
N2	9.5	0.39	0.153a	10.4	0.37	0.145ab
Rp2	10.8	0.40	0.153a	10.8	0.41	0.160a
Re	7.3	0.33	0.130b	7.6	0.30	0.123c
K1	7.6	0.36	0.142ab	7.6	0.35	0.140b
K2	11.3	0.37	0.146a	11.6	0.33	0.121c
Mean	9.1	0.38	0.146	9.4	0.35	0.138

N = average sample size, P = proportion of polymorphic loci within each population, He = gene diversity; He values of the same year followed by same letter are not significantly different, ($P < 0.05$); means of P and He of the two years were tested by the t test and found not significantly different

Table 2 Spatial and temporal genetic diversity estimates calculated by means of analysis of variance (AMOVA) and by means of coancestry based on 1,545 AFLP bands in six populations and 2 years

Year	Source of variation	df	Sum of squares	Variance	Percentage of variation	Coancestry coefficient (CL)
1988	Between populations	5	1231.0	14.65	12.70*	$\theta = 0.1243(0.1121–0.1368)$
	Within populations	54	5433.5	100.62	87.29*	
	Total	59	6664.6	115.27	100.00	
2002	Between populations	5	1586.8	21.85	17.92*	$\theta = 0.176(0.1610–0.1915)$
	Within populations	54	5406.6	100.12	82.08*	
	Total	59	6993.4	121.98	100.00	
All pops. in both years	Between years	1	91.5	−3.28	−2.84	$\theta-p = -0.0371(-0.0404 \text{ to } -0.0339)$
	Between populations within years	10	2817.8	18.25	15.82*	$\theta-s = 0.11920(0.1083–0.1301)$
	Within populations	108	10840.1	100.37	87.02*	
	Total	119	13749.5	115.34	100.00	

Analysis of variance was carried out using Arlequin Ver. 2.000; Coancestry coefficients were obtained from GDA software

AMOVA, Excoffier et al. (1992)

GDA (Genetic Data Analysis; Lewis and Zaykin 2001) version 1.1. CL = 95% confidence limit by bootstraping across loci 5,000 cycles

*The probability, based on 1,023 permutations, of a random value to be > = than the observed percentage of variation value = 0 ± 0.00

Table 3 Genetic distances (Nei 1978) among six wild emmer populations in the years 1988 and 2002 (all monomorphic and polymorphic loci were considered)

	N1 88	N2 88	Rp2 88	Re 88	K1 88	K2 88	N1 02	N2 02	Rp2 02	Re 02	K1 02
N2 88	0.021										
Rp2 88	0.026	0.019									
Re 88	0.033	0.025	0.021								
K1 88	0.035	0.027	0.031	0.020							
K2 88	0.060	0.058	0.055	0.068	0.046						
N1 02	**0.005**	0.031	0.033	0.038	0.041	0.078					
N2 02	0.016	**0.011**	0.026	0.030	0.032	0.069	0.022				
Rp2 02	0.025	0.018	**0.005**	0.024	0.029	0.052	0.032	0.022			
Re 02	0.035	0.025	0.022	**0.002**	0.020	0.068	0.039	0.031	0.022		
K1 02	0.033	0.029	0.029	0.021	**0.000**	0.041	0.041	0.036	0.027	0.020	
K2 02	0.076	0.071	0.075	0.086	0.058	**0.007**	0.096	0.083	0.066	0.084	0.053

N1, N2, and Rp2 were clustered in the first group, Re, and K1 in the second group, and K2 in the third group, being remote from the other populations.

Discussion

Genetic diversity

Spatial

The partition of the genetic diversity within and among the six studied populations of wild emmer wheat was quantified by the measures of frequency of polymorphic loci (*P*) and gene diversity (*He*) (Table 1). The diversity values that were detected by the 1,545 AFLP bands were much higher than values obtained from the same populations by Nevo et al. (1991) and Li et al. (2001) using allozyme markers, by Felsenburg et al. (1991) using storage proteins, and by Huang et al. (1999) using RFLP markers. However, since the AFLP technique assumes that all variation is biallelic, the diversity values obtained by AFLP analysis are artifactually higher than those that are based on multiallelic systems. The frequency of polymorphic loci varied to some extent between populations and to a lesser extent between years. The populations studied are located in the center of wild wheat distribution in Israel and the high polymorphism characterizing them is in accordance with the data of Nevo and Payne (1987) and Levy and Feldman (1988), who, studying HMW glutenin genes, found that central

populations of wild emmer, like those of Ammiad, were very polymorphic. A similar high level of polymorphism was found in central populations of wild barley, *Hordeum spontaneum*, with respect to polymorphism of hordein genes (Doll and Brown 1979; Nevo et al. 1983).

The parameter gene diversity (*He*; Nei 1973), takes into account the frequency of the alleles and consequently, their contribution to the diversity of the locus. Hence, it is the most suitable estimation of genetic diversity. The average gene diversity found in the six studied populations was 0.146 in the year 1988 and 0.138 in the year 2002 (Table 1). There were significant differences in gene diversities between populations but not between years.

Considering the structuration of genetic diversity in annual, self-pollinating species, Hamrick and Godt (1990) claimed that in annual, self-pollinating species that do not disperse genes through pollen or seeds to a long distance, most of the genetic variation is between- rather than within-populations. Similarly, Nybom (2004) assumed that long-lived outcrossing species retain most of their genetic variability within populations while annual, self-pollinating species allocate most of the genetic variability between populations. However, previous studies with allozyme markers in central populations of wild emmer wheat (Nevo and Beiles 1989; Nevo et al. 1991) showed that a high degree of genetic diversity is partitioned within, rather than between, populations. Also, in central populations of wild barley it was found that the degree of diversity is greater within- than between-populations (Nevo et al. 1979, 1986; Dawson et al. 1993; Baum et al. 1997; Turpeinen et al. 2001, 2003). Our finding that the within-populations gene diversity (0.825) was much larger than the between-populations gene diversity (0.175) is in accord with these studies. On the other hand, in peripheral and marginal populations of wild emmer wheat the within-populations genetic diversity is much smaller than the between-populations diversity (Nevo 1998; Nevo and Payne 1987; Nevo and Belies 1989; Levy and Feldman 1988; Jaradat 2001a, b). Peripheral and marginal populations grow on a relatively more homogeneous environment and are isolated or semi-isolated from the central populations and there is little, if any, gene flow between the populations. Consequently, the genetic differences between them are large. Such isolated populations however, may harbor unique alleles (Nevo 1998, 2001). Previous studies (Hamrick and Allard 1972; Hamrick and Holden 1979) already showed that polymorphism is more likely to be maintained in a more heterogeneous environment; the higher the spatial or temporal environmental heterogeneity, the larger the genetic polymorphism maintained in the population (Hedrick et al. 1976; Spieth 1979). Our studies showed that the highest genetic diversity occurred in populations N1, N2, and Rp2 while the lowest genetic diversity was found in Re, K1, and K2. Populations N1, N2, and Rp2 grow on the northern slope or on the ridge,

assumed to be relatively mesic habitats and therefore, more heterogeneous, while Re grows on eastern slope and K1 and K2 on southern slopes, relatively xeric habitats and therefore, less heterogeneous. This is in accordance with the findings of Nevo (1998) that the diversity measures were more variable in mesic environments than in populations in xeric environments.

Temporal

Plants derived in the years 1988 and 2002 from the same sampling point exhibited non-significant differences in genetic diversity estimates (Table 1). The relatively low values of temporal genetic diversity estimates of the six studied populations are in spite of the differences in the annual rainfall in the preceding growing seasons of 1986–1987 and 2000–2001 (Supplementary Materials Table S2) that may affect the ratio between the various genotypes at the beginning of the studied years. Similar results, indicating low values of temporal genetic diversity, were obtained in 5-year period studies of wild emmer populations in Ammiad (Nevo et al. 1991; Felsenburg et al. 1991) and in Jordan (Jaradat 2001a, b). In our studies, 14 years of difference between the first sampling and the second did not yield larger values of temporal genetic diversity.

The positive and highly significant correlation between the θ values of the populations of the year 1988 with those of 2002 (θ of 88 and θ of 02) indicates similar trend of spatial differentiation in the 2 years.

There are three major factors that might contribute to the small temporal changes: mutations, sampling of different genotypes that accommodate the same sampling point, and migration of different genotypes from neighboring sites (newcomers). The effect of mutations can account only for a small number of changes. Migration of genotypes from a neighboring site can also account for some of the changes though pollen and seeds are not usually dispersed far from their mother plant (Golenberg 1988). Therefore, a reasonable explanation for the changes between these 2 years is that several genotypes grew around many of the sampling points and that the relative frequency of every genotype changed between the years 1988 and 2002 due to variation in environmental factors such as fluctuations in the amount and distribution of the annual rainfall. Such demographic fluctuations were observed in Ammiad during the years 1985–1988 (Noy-Meir et al. 1991). Changes in plant density in different years may result in some turnover at the local scale. Recolonization of "empty" spaces due to relative drought in previous year(s) may be due to the germination of dormant seeds of other genotypes. This small amount of temporal turnover is consistent with the degree of genotype shifts between years at individual sampling points (Felsenburg et al. 1991: Nevo et al. 1991).

Classical population genetics deals with genotype frequencies in a population as determined by the processes of mutation, selection, gene flow and drift, while assuming that the number of individuals in the population is constant from generation to generation. The number of plants in populations of annuals often varies greatly from year to year, particularly in unstable environments. The analysis of genotype frequencies and their explanation in term of processes such as selection must take into account the dynamics of population size (Jain 1975; Harper 1977). Environmental factors that have large effects on the fluctuation of demographic processes in space and time, and thus on population dynamics, are often important selective forces affecting genetic composition and its spatio-temporal variation (Noy-Meir et al. 1991).

Genetic divergence

Higher similarity prevailed in each population between the two years than between populations of each year as evident in Table 3 and supplementary Materials Figure S2. This also suggests that spatial effect on genetic divergence is larger than temporal effect.

Relative importance of spatial and temporal factors in maintaining population polymorphism

The relative genetic diversity and the divergence estimates between the six populations were significantly larger than the estimates between years. Our findings are relevant to geographically closely related wild emmer populations and to temporal effect during 14 years. The results may be different if the effect of longer period would be studied. However, similar evidence that the spatial diversity and divergence are larger than the temporal ones in wild emmer populations that are not geographically closely related were obtained by Nevo et al. (1991) using allozymes, Felsenburg et al. (1991) using HMW-glutenins, and Jaradat (2001a, b) using allozymes and HMW-glutenins. Hence, spatial factors account for the main portions of the population genetic variation, suggesting strong provenance specificity (Horovitz and Feldman 1991), and definitely are more important than temporal ones in maintaining genetic variation in wild emmer populations. Similar results were obtained also in populations of wild barley (Turpeinen et al. 2003). These results support the prediction of Karlin (1982), on the basis of theoretical calculations, that spatial variation is more effective in protecting a polymorphism in a population than temporal variation. For a longer period however, this may not be necessarily true.

The genetic variation found in natural populations of wild emmer wheat, as well as in wild barley, is very high, much higher than that of domesticated wheat (e.g., Feldman

and Millet 1995; Feldman 2001; Huang et al. 1999). Hence, genes existing in wild wheat may have direct breeding potential and should be conserved. Detailed information on the genetic structure of populations of wild crop relatives and its relationship to spatial and temporal factors is imperative for the efficient maintenance of their germplasm. It provides a scientific base for rational guidelines for a detailed monitoring of variation patterns in space and time. The information obtained in this work can assist in designing a strategy for the in situ conservation of the genetic resources of wild emmer.

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