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SPATIAL AND TEMPORAL GENETIC STRUCTURE OF WILD EMMER WHEAT IN JORDAN. II. HIGH-MOLECULAR-WEIGHT GLUTENINS AND ALLOZYMES

A.A. JARADAT†
Jordan University of Science and Technology, P.O. Box 3629, Irbid, Jordan

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ABSTRACT

A study was designed to sample and characterize 12 populations of wild emmer wheat in Jordan at the high-molecular-weight (HMW) glutenin and allozyme levels. Data were collected and individual plants were sampled from 207 permanent sampling points in three central, three peripheral, and six marginal populations during all or part of a five-year study period. Patterns of variation, based on four HMW-glutenin and 36 allozyme loci, were used in characterizing these populations. Polymorphisms, based on HMW-glutenins and allozymes, decreased with increasing aridity of the collection site. A large portion of the HMW-glutenin(61.1%) and allozyme (62.7%) variant alleles were localized. Gene differentiation estimates, based on HMW-glutenins and allozymes among (60.8 and 65.8%, respectively) and within (39.2 and 34.2%, respectively) populations, were high and comparatively similar; however less variation was partitioned among the Jordanian populations when compared to populations in the more mesic parts of the Fertile Crescent. Environmental heterogeneity may have been involved in generating significant spatial structuring in these populations at the HMW-glutenin and allozyme levels. A gradual increase in allozymic monomorphism with aridity suggests that polymorphism is more likely to be found in heterogeneous environments. Changes in population size and total loss of small populations are warning signs that these populations may be vulnerable. Results of the present study are expected to complement the existing body of knowledge on the species and enhance the understanding of its genetic differentiation and evolution across large parts of the Fertile Crescent. The preservation of the endangered, and yet valuable, genetic diversity present in Jordanian wild emmer wheat populations, as an integral part of a holistic regional approach to biodiversity conservation, is urgent.

INTRODUCTION

Wild plant populations adapt, at the morphological (Allard et al., 1968), developmental (Jaradat and Humeid, 1990), allozyme (Nevo, 1987; Nevo et al., 1988a,b, 1991; Nevo and Beiles, 1989), and seed storage protein (Levy and Feldman, 1988; 1989; Nevo, 1983; Felsenburg et al., 1991) levels, to habitat variation to meet the local macro- and microenvironmental conditions. This adaptation potential in wild emmer wheat explains its distribution over a large ecological amplitude in the Fertile Crescent.

A few studies (Felsenburg et al., 1991; Nevo et al., 1991) have addressed the long-term fluctuation in the range and pattern of genetic variation, both within and among populations of wild emmer wheat, as affected by yearly climatic changes. Information from such long-term studies is necessary for devising better strategies for the in situ conservation and ex situ sampling of the

†Present address: Plant Genetic Resources Program, International Center for Biosaline Agriculture, ICBA P.O. Box 14660, Dubai, UAE.
E-mail: aajaradat@hotmail.com

genetic variation available in the species.

This paper reports on natural wild emmer wheat collections from 12 main populations in Jordan during part or all of the collecting period (1987–1991) and addresses spatial and temporal variability in diversity indices calculated for four HMW-glutenin and 36 allozyme loci.

MATERIALS AND METHODS

HIGH-MOLECULAR-WEIGHT GLUTENIN POLYMORPHISM

Seeds from a total of 1273 individual emmer wheat plants were used in this study. These represented the five-year collections from 207 permanent sampling points in 12 populations (see Jaradat, this issue). Endosperm storage-protein subunits were extracted from kernels, excluding the embryo. The sample buffer solution and fractionation procedure were similar to those described by Galili and Feldman (1983), while the assignment of subunits of high-molecular-weight glutenin loci and allele designation followed those of Felsenburg et al. (1991). Bread wheat cultivar Chinese Spring, with known HMW glutenin subunits, was used in each gel as a reference.

ALLOZYME POLYMORPHISM

A total of 589 individual wild emmer wheat plants were assayed electrophoretically for genetic variation at 36 shared loci. These plants represented all 207 sampling points and 12 populations during the 1988 and 1991 sampling seasons only. The tissue preparation procedures and horizontal starch gel electrophoresis techniques described by Nevo and Beiles (1989 and references therein) were followed. Locus and allele designations of Nevo et al. (1982) were used in the present study.

STATISTICAL ANALYSIS

Allele distribution. The classification developed by Marshall and Brown (1975) and used by Nevo et al. (1991) was adopted to classify each allele (allozymes and HMW-glutenin loci), based on its frequency in each population and the number of populations it appeared in, into (1) common widespread, (2) common sporadic, (3) common localized, (4) rare widespread, and (5) rare localized.

Gene diversity statistics. Allelic frequency data were used to compute gene diversity statistics (Nei, 1973, 1987) i.e., gene diversity at each locus, *H*; average gene diversity over all loci, H_e; proportion of polymorphic loci, *P*; average number of alleles per locus, *A*; and effective number of alleles per locus, *Ne*.

Gene diversity, introduced under the name "polymorphic index" by Marshall and Allard (1970) and as "gene diversity" by Nei (1973), is defined at a single locus as one minus the sum of squared allelic frequencies. Generally, gene diversity is a more appropriate measure of variation than heterozygosity, especially in predominantly selfing species of plants. These species may contain a great deal of variation, but very few heterozygous individuals.

The algorithm used to compute genetic diversity within and between populations was based on the GENESTAT program (Whitkus, 1985) and the program GDA (Lewis and Zaykin, 1999). The following gene diversity statistics were computed for each locus: total gene diversity, *Ht*; average gene diversity within populations, *Hs*; average gene diversity between populations, *Dst*; and gene diversity between populations relative to total gene diversity, *Gst* (Nei and Chesser, 1983; Nei, 1987). Finally, the standard genetic distances (Nei, 1987) were calculated for each population.

Analysis of variance and estimation of variance components. The Minimum Variance Quadratic Estimator form [MIVQUE(0)]of the Maximum Likelihood (ML) estimation method of STATISTICA (StatSoft, 1998) was employed for analysis of variance and estimation of variance components. The Sattertwaite method of denominator synthesis, as described in StatSoft (1998), was used to test for significance of the respective effect of interest in the analysis of variance. This method finds the linear combinations of sources of random variation that serve as appropriate error terms. Asymptotic tests of significance of MEVQUE(0) variance component estimates were constructed from the final iteration of the solution.

RESULTS

HMW GLUTENINS

Allelic frequencies. Allelic frequencies computed for all four HMW-glutenin loci are presented in Table 1. The number of alleles, including the null allele *a*, was four at *Glu-A1-1* (*a, k, o*, and *t*, in increasing order of molecular weight, according to Felsenburg et al., 1991), two at *Glu-A1-2* (*a* and *l*), five at *Glu-B1-1* (*a, c, j, l*, and *u*), and seven at *Glu-B1-2* (*a, g, k, m, n, p* and *s*).

Allele *t* at the *Glu-A1-1* locus appeared with the highest frequency (74.08%), while allele *k* was the least frequent (3.18%). The null allele (*a*) in *Glu-A1-2* was widely prevalent (96.3%). This variation in allelic frequencies was reflected in the mean gene diversity of these two loci. *Glu-A1-1* was much more polymorphic (mean gene diversity = 0.491, range 0.000–0.587) than *Glu-A1-2* (mean = 0.095, range 0.0217–0.262). How-

Table 1
Alleles of four HMW glutenin loci found in wild emmer wheat plants collected from 12 natural populations in Jordan, their frequencies, diversity indices, and tests of significance among populations

Locus	allele	Frequency	Average *H*	Range *H*	% Sig. diff. among populations N = 66	% Variance due to populations	Asymptotic *p*
Glu-A1-1			0.491	0.000–0.587	44	35.8	0.077
	a	0.071					
	K	0.032					
	o	0.156					
	T	0.741					
Glu-A1-2			0.095	0.022–0.262	17	6.9	0.104
	a	0.963					
	L	0.037					
Glu-A			0.319	0.221–0.428	56	34.0	0.05
Glu-B1-1			0.537	0.438–0.590	3	4.8	0.157
	a	0.005					
	C	0.024					
	J	0.064					
	L	0.172					
	u	0.735					
Glu-B1-2			0.696	0.512–0.755	18	4.2	0.231
	a	0.042					
	g	0.020					
	k	0.057					
	m	0.545					
	n	0.032					
	p	0.056					
	S	0.249					
Glu-B			0.681	0.571–0.778	56	34.0	0.05
Average			0.455				

ever, average polymorphism for the compound locus *Glu-A* was 0.3186 (range 0.221–0.428).

The allele *Glu-B1-1u* appeared with the highest frequency (73.5%), followed by allele *l* (17.22%), whereas alleles *j, c,* and *a* at *Glu-B1-1* appeared with decreasing frequency, in this order. Mean gene diversity for this locus was 0.537 (range 0.438–0.590), which was higher than gene diversity for the previous loci (i.e., *Glu-A1-1* and *Glu-A1-2*), however, lower than gene diversity of the most diverse (0.696) locus *Glu-B1-2*. The latter displayed 7 alleles with frequencies ranging from 54.5% for allele *m* to 1.96% for allele *g*.

Alleles were assigned to four of the five frequency groups; none fitted the "common sporadic" category. The following classification is a summary (without detail) of allelic frequency across populations. Four alleles (*Glu-A1-1t, Glu-A1-2a, Glu-B1-1u,* and *Glu-B1-2m*) were classified as common widespread alleles. Three alleles (*Glu-A1-1o, Glu-B1-1l,* and *Glu-B1-2s*) were classified as common localized. Eight alleles (*Glu-A1-1a, Glu-A1-1k, Glu-A1-2l, Glu-B1-1a, c, and Glu-B1-2g, k, n*)were classified as rare localized alleles. Three alleles were classified as rare widespread (*Glu-B1-1j* and *Glu-B1-2a* and *p*). All null alleles were classified as rare localized (*Glu-A1-1a* and *Glu-B1-1a*) or rare widespread (*Glu-B1-2a*), except *Glu-A1-2,* which was classified as a common widespread allele (Fig. 1).

Intragenomic allelic patterns. Five of the eight possible intragenomic allelic combinations were identified at the *Glu-A1* compound locus. The highest frequency (73.32%) was found for the combination *Glu-A1-1t* and *Glu-A1-2a*, while the lowest (1.2%) was found for the combination *Glu-A1-1k* and *Glu-A1-2l*. Seventeen of the possible 35 intragenomic combinations were formed by alleles at the *Glu-B1* compound locus. The highest frequency (38.7%) was found for the combination *Glu-*

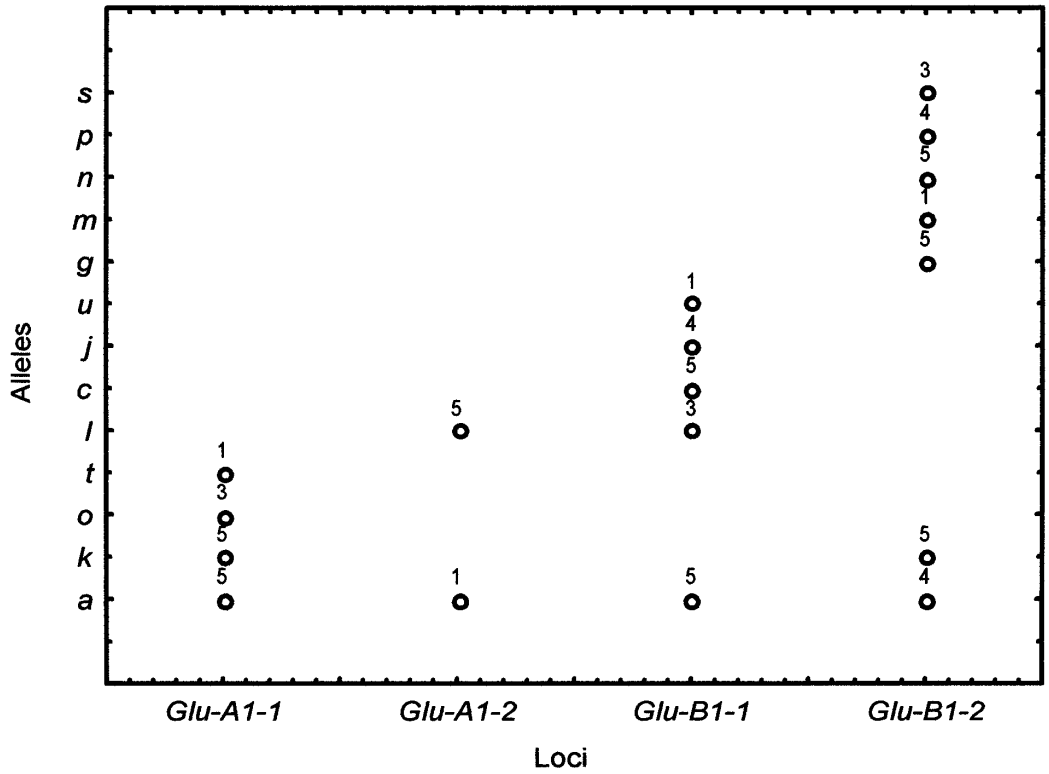

Fig. 1. Frequency groupings of18 HMW-glutenin subunit alleles at four loci, scored on wild emmer wheat plants in 12 natural populations in Jordan. The classification follows Marshall and Brown (1975) and is based on frequencies per population and the number of populations in which an allele appears. 1 = common widespread; 2 = common sporadic; 3 = common localized; 4 = rare widespread; 5 = rare localized.

B1-1u and *Glu-B1-2m*, whereas the lowest frequency (0.48%) was found for the combination *Glu-B1-1l* and *Glu-B1-2g*.

Intergenomic allelic patterns. A small fraction (21 or 24.7%) of the total possible number of intergenomic combinations (five in *Glu-A1* and 17 in *Glu-B1*) were formed by alleles at both compound loci. The highest frequency (26.2%) was found for the intergenomic combination of alleles *t* and *a* at *Glu-A1-1* and *Glu-A1-2*, respectively, and alleles *u* and *m* at *Glu-B1-1* and *Glu-B1-2*. The lowest frequency (0.96%) was recorded by the intergenomic combination [*Glu-A1-1t*/*Glu-A1-2l* and *Glu-B1-1u*/*Glu-B1-2g*].

Gene diversity analysis. Significant differences in gene diversity were found among populations for both the *Glu-A1-1* and *Glu-A1-2* loci, however the magnitude of these differences was higher for the former than the latter locus. There were 44% significant pairwise differences among the 66 possible pairwise population comparisons for *Glu-A1-1*, as compared to 17% for *Glu-A1-2*. Nevertheless, differences among populations explained 35.8% and 6.9% of total variation in mean gene diversity for *Glu-A1-1* and *Glu-A1-2*, respectively; both variance components were not significant at $p < 0.05$. Average gene diversity for the compound locus *Glu-A* was 0.3186 (range 0.221–0.428). Differences in average gene diversity for this compound locus among populations were considerable; more than half (56%) of the 66 pairwise population comparisons were significant, and differences among populations accounted for 34% ($p < 0.05$) of total variance in gene diversity of the compound locus.

The B-genome loci exhibited a higher polymorphism than those of the A-genome, with *Glu-B1-2* as the most polymorphic locus (mean = 0.697 and range 0.512–0.755), followed by *Glu-B1-1* (mean = 0.537; range 0.438–0.590). Alleles at the *Glu-B1-1* locus varied widely in their frequency; the lowest frequency (0.490) was scored for allele *a* (the null allele), and the highest (0.735) for allele *u*. Average polymorphism for the *Glu-B1-1* locus was 0.537 (range 0.438–0.590). This narrow range reflects the very few pairwise population mean differences in average polymorphism at this locus; only two (~3%) of the 66 pairwise population comparisons

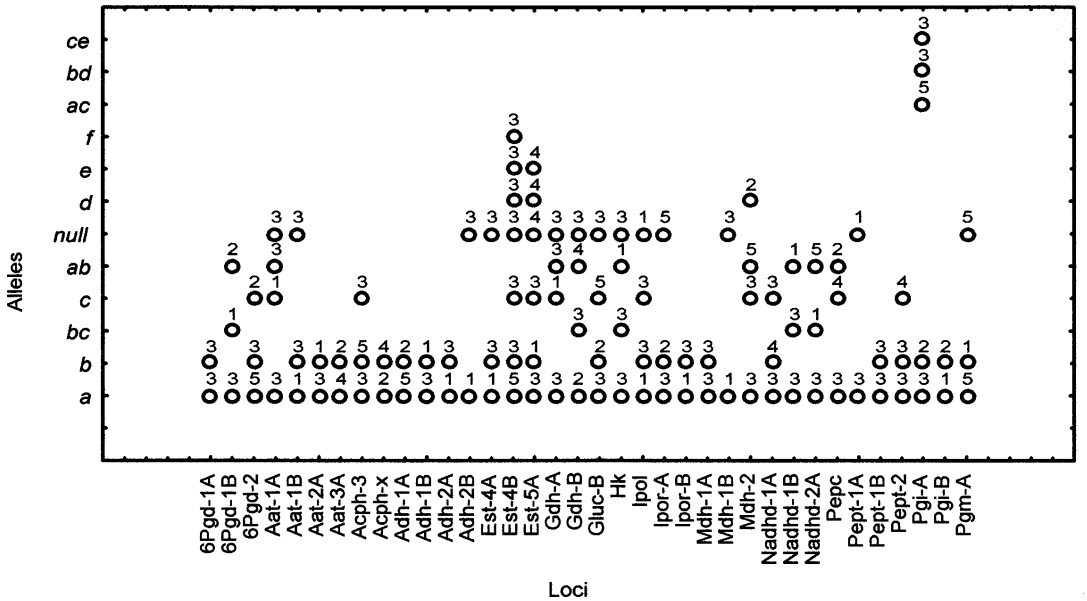

Fig. 2. Frequency groupings of 110 allozyme alleles at 36 loci, scored on wild emmer wheat plants in 12 natural populations in Jordan. Classification as in Fig. 1.

were significant, and the differences among populations explained a very small portion (4.8%; $p < 0.157$) of total variation in polymorphism at this locus.

Average polymorphism at the *Glu-B1-2* locus was 0.696 (range 0.512–0.755). This high value was the result of a large number of alleles (seven compared to four alleles at the *Glu-B1-1* locus, four at *Glu-A1-1,* and only two at *Glu-A1-2*) and of the contribution of most alleles to the observed high polymorphism. Allele *m* appeared with the highest frequency (0.545) among alleles at the *Glu-B1-2* locus, followed by allele *s* (0.249). The remaining alleles (*a, g, k, n,* and *p*) ranged in frequency from 0.020 to 0.057.

Significant differences among populations in *Glu-B1-2* were few (12 out of 66 pairwise comparisons, or about 18.0 %); these differences accounted for a small and nonsignificant portion (4.2%; $p < 0.231$) of the total variance in *Glu-B1-2* polymorphism. However, when polymorphism of the compound *Glu-B* locus was considered (average 0.681; range 0.571–0.778), more than half (56%) of the 66 pairwise population comparisons were highly significant; these differences among populations accounted for 34.0% of total variance ($p < 0.05$) in *Glu-B* polymorphism.

Partitioning of total gene diversity. Total gene diversity (*Ht*) was partitioned into its components: average gene diversity within populations, *Hs*, average gene diversity among populations, *Dst*, and the value of gene diversity among populations relative to total gene diversity, *Gst*, were calculated. Total gene diversity was estimated as 0.522; a relatively smaller portion (39.2%) of this diversity was partitioned within populations (*Hs* = 0.317), as compared to 60.8% of total gene diversity partitioned among populations. However, when individual loci were considered, *Ht* and *Hs* for *Glu-A1* and *Glu-B1* were 0.490 and 0.289, and 0.554, and 0.345, respectively. *Gst*, on the other hand was 41.0% for *Glu-A1* and 34.5% for *Glu-B1*, thus indicating a substantial gene differentiation between populations.

Spatial-temporal variability. Spatial and temporal changes in frequencies of all four glutenin loci were tested using a chi-square test. The chi-square tests indicate that *Glu-A1-1* experienced significant frequency changes across populations, (chi-square = 47.3, df = 33; $p < 0.0509$), whereas *Glu-A1-2* reflected temporal changes (chi-square = 7.56, df = 2; $p < 0.022$) over the study period. On the other hand, spatial and temporal factors were highly influential in altering frequencies of certain alleles but not others across populations and over the study period (data not presented).

ALLOZYME SYSTEMS

Allele distribution. The analogous classification developed by Marshall and Brown (1975) was used for regional analysis of allele distribution. Data from both years did not differ significantly and were combined for each of the 12 populations and utilized in this analysis. The 110 alleles found in 36 allozyme systems were

Table 2
Mean genetic indices based on 36 allozyme systems in 12 natural wild emmer wheat populations in Jordan during two growing seasons (1988 and 1991)

Location	Gene diversity, H_e	Polymorphism, *P*-5%	Effective number of alleles, *Ne*	Polymorphic loci	
				Number	Percent
Ishtafina	0.113	0.441	1.138	2	5.50
Zobia	0.147	0.498	1.179	5	13.9
Zaatari	0.162	0.512	1.195	5	13.9
Afana	0.119	0.354	1.147	0	00.0
Natfeh	0.096	0.297	1.106	5	13.9
Sakhra	0.067	0.251	1.076	11	30.5
Ibbien	0.077	0.245	1.089	12	33.3
Jarash	0.047	0.162	1.055	17	47.2
Jraineh	0.029	0.114	1.034	19	52.7
Naor	0.034	0.154	1.036	16	44.4
Samad	0.062	0.194	1.064	13	36.1
Umamad	0.059	0.211	1.069	14	38.9
Overall mean	0.0845	0.2114	1.064	10	27.8
% Sig. diff. among populations, N = 66	45.45	46.96	43.93		
%Variance due to populations	57.16	58.9	57.2		
Asymptotic *p*	0.001	0.001	0.001		
% Sig. diff. among years	0.0	0.0	0.0		

classified (Fig. 2) as follows: 20 common widespread; 12 common sporadic; 58 common localized, 9 rare widespread, and 11 rare localized alleles. A total of 69 alleles (62.7%) were classified as localized and 29 alleles (26.4%) as widespread. The nonrandom allelic differentiation of the localized alleles indirectly supports the highly significant differences among populations in gene diversity and polymorphisms (see later).

Gene diversity. Genetic data (gene diversity, *He*, the 5% polymorphism criterion, *P-5%*, and effective number of alleles, *Ne*) for each of the 12 populations and the overall average are presented in Table 2. Gene diversity averaged 0.0845 and ranged from 0.029 for the marginal and xeric population Jraineh to a high value of 1.620 for the central and mesic population Zaatari. Populations differed significantly in their estimated *He* values, and there were 30 (45.45%) pairwise significant differences among populations. Consequently, differences among population accounted for a large (57.16%) and highly significant ($p < 0.001$) portion of total variance in gene diversity.

Polymorphism. Large and significant differences were detected in *P-5%*. Average polymorphism was 0.211 and ranged from 0.114 in the least diverse population, Jraineh to 0.512 in the most diverse population, Zaatari. As was the case for gene diversity, significant differences among populations were high (46.96%), and differences among populations accounted for a large (58.9%) and highly significant ($p < 0.001$) portion of polymorphism estimates.

Effective number of alleles. On average, the effective number of alleles (*Ne*) over populations and allozyme systems was 1.064. Populations differed significantly from each other in their *Ne* estimates (range 1.034 in Jraineh to 1.195 in Zaatari); There were 43.93% pairwise significant differences among the 66 pairwise comparisons; this was reflected in the highly significant ($p < 0.001$) portion of total variance (57.2%), accounted for by differences among populations.

Temporal variation in gene diversity, polymorphism, and effective number of alleles was not significant. A clear trend was observed for all three genetic indices (*He, P-5%,* and *Ne*) when central, peripheral, and marginal populations were considered. *He* estimates for central, peripheral, and marginal population were 0.140, 0.094 and 0.051, respectively. *P-5%* averaged 0.483 for central populations, 0.3 for peripheral, and 0.18 for marginal populations. These sharp declines in *He* and *P-0.05* were not paralleled by a sharp decline in the effective number of alleles. *Ne* was estimated as 1.170, 1.109, and 1.057 for central, peripheral, and marginal populations, respectively.

Genetic differentiation within and among populations. Total gene diversity (*Ht*), based on 36 allozymes, was partitioned into its components: average gene diversity within populations, *Hs*, and average gene diversity

among populations, *Dst*, and the value of gene diversity among populations relative to total gene diversity, *Gst*, was calculated according to Nei (1973; 1987). Average values obtained for both years did not differ significantly from each other and they were pooled for the analyses of genetic differentiation within and among populations. The average total gene diversity over all 36 allozyme loci in 12 populations was 0.163, whereas the average gene diversity among populations was 0.107. The average relative differentiation, i.e., *Gst*, among the 12 populations was 0.342, i.e., a large part of allozymic variation (65.8%) was within populations, and 34.2% was among populations (average *Dst* = 0.0557). Four loci (*Pgi*-B, *Nadh*-1B, *6Pgd*-2, and *Est*-5A, in decreasing order, average *Dst* = 0.0954) displayed the highest gene diversities between populations.

DISCUSSION

The multidisciplinary study carried out in 12 populations of wild emmer wheat in Jordan had one major objective to address, namely: to draw a detailed spatial-temporal map of genetic diversity of the species and to quantify the genetic structure and differentiation of the species in a major part of its distribution. This effort should add to an understanding of genetic differentiation and evolution of the species across large parts of the Fertile Crescent region and advance in situ conservation and potential utilization of wild emmer wheat as a valuable genetic resource in wheat improvement.

In this context, results of this study will be discussed in relation to the geographic range, regional distribution, breeding system, and seed dispersal mechanism of wild emmer wheat. Furthermore, the buffering capacity of the "seed bank", i.e., seed accumulated in the soil over the years, and its role in reducing the fitness uncertainty generated by the "typical" cyclical or random Mediterranean environments, will be addressed.

HIGH-MOLECULAR-WEIGHT GLUTENINS

Polymorphisms. Seed storage proteins (e.g., HMW-glutenins) have been studied intensively not only because they are important nutritionally, but because they provide a biological model system for the temporal and spatial regulation of gene expression (Nevo, 1983; Gepts, 1990). Polymorphisms in seed storage proteins have been identified in several wild and cultivated species, including wild emmer wheat. Their high levels of polymorphism in a number of cultivated (e.g., *Phaseolus vulgaris* L.) and wild (e.g., *Hordeum spontaneum* C. Koch and *dicoccoides* wheat) species contrast with the relatively lower level of allozyme diversity. In wild emmer wheat, for example, the mean number of alleles per locus, (A = 2.5), the proportion of polymorphic loci per population, (p = 0.77), and gene diversity, (He = 0.36) for HMW-glutenins were, respectively, 1.9, 3.0, and 3.7 times higher than those for allozymes (Nevo et al., 1982; Nevo and Payne, 1987). However, a disadvantage of these loci is that they cover only a small portion of the genome when compared with allozymes.

High-molecular-weight glutenins and other seed storage proteins offer a number of advantages as genetic markers in population genetic studies. They can be analyzed at a low cost by electophoresis and reveal high levels of diversity. This diversity, expressed in electrophoretic patterns, appears to be mostly genetically controlled, with little if any environmental effects, and appears to have a unique origin. The genetic control of qualitative variation in HMW-glutenins is simple and involves a limited number of loci whose location is known; the various alleles are codominant, therefore the genotype can be directly determined from the banding pattern.

Since frequencies of HMW-glutenin alleles vary widely (e.g., 0.49–96.3% in this study; 0.09–96.12% in Felsenburg et al., 1991; 0.0–88.0% in Ciaffi et al., 1993b), the number of alleles per locus does not express genetic diversity accurately. Gene diversity (Nei, 1973, 1987; Weir, 1996), introduced earlier under the name "polymorphic index" (Marshal and Allard, 1970) was suggested as a more suitable parameter; it takes into account the frequency of each allele and its contribution to the diversity of the locus.

Apparently, polymorphism of the HMW-glutenins in this study was affected by the number of active genes, the number of alleles within each active gene and their frequency, and the inter- and intragenomic combinations among the existing alleles (Levy et al., 1988). However, the inclusion of six marginal populations in this study highly reduced the overall average for gene diversity (0.354), polymorphism (P-5% = 0.763), mean number of alleles per locus (A = 2.013), and mean effective number of alleles per locus (Ne = 1.18), although none of the 12 population was monomorphic for all loci. Gene diversity was almost equal to an estimate of 0.357, based on 231 genotypes representing 11 wild emmer wheat populations from Israel (Nevo and Payne, 1987). However, the Jordanian populations displayed a slightly lower number of alleles per locus (2.013 vs. 2.5) and a slightly lower polymorphism (0.763 vs. 0.773). These values were 20–25% higher when calculations were based on central and peripheral populations only (data not presented). On the other hand, the gene diversity value averaged over loci and populations was slightly higher than an estimate reported by Felsenburg

et al. (1991) for the central Ammiad population in Israel (0.454 vs. 0.410), however, there were some discrepancies when individual gene diversity values were contrasted. This finding supports an earlier conclusion (Levy and Feldman, 1988) where, in accordance with Vavilov's theory that centers of distribution are also centers of variation, marginal populations were found to be less polymorphic than central ones.

Ciaffi et al. (1993a,b) used a part of the Jordanian wild emmer wheat collection in a comparative study with Turkish germplasm. These researchers reported relatively higher values for mean number of alleles per locus (A = 3.52), mean effective number of alleles per locus (Ne = 2.83), polymorphism (P-5% = 0.85), and genic diversity (He = 0.553), although one of their nine populations (Zaatari) was found to be totally monomorphic. Ciaffi and coworkers attributed the high estimates to the greater number of allelic variants (15 and 19, at the *Glu-A1* and *Glu-B1* compound loci, respectively), and of genotypes per population, considered in their study.

Results reported in Table 1 support earlier findings (e.g., Levy and Feldman, 1988; Felsenburg et al., 1991) in which B-genome loci were found to be more polymorphic than A-genome loci, of which *Glu-A1-2* was the least variable. Most probably, the reduced polymorphism of the A-genome loci (Galili and Feldman, 1983; Levy et al., 1988; Felsenburg et al., 1991) reflects the nonrandom inactivation of HMW-glutenin genes affecting *Glu-A1-2* (H = 0.095) to a greater extent than *Glu-A1-1* (H = 0.491), *Glu-B1-1* (H = 0.537) and *Glu-B1-2* (H = 0.696).

Gene differentiation among and within populations. Impressive high glutenin diversity estimates, similar to those reported for allozyme systems, among and within populations were reported for wild emmer wheat from Israel (Nevo and Payne, 1987; Felsenburg et al., 1991; Pagnotta et al., 1995a,b) and Jordan (Ciaffi et al., 1993b). In this study, however, the level of gene differentiation among populations, although substantial, was lower than estimates reported by other researchers. The discrepancy in *Gst* values (52% and 63% reported by Nevo and Payne (1987) and Pagnotta et al. (1995b), respectively, as compared to 39.2% in this study) could have been brought about by a concomitant increase in population size and variation leading to an increase in the among-population variation (Ellstrand and Elam, 1993). Nevertheless, the *Gst* estimates indicate that population divergence in wild emmer wheat does not always conform to the isolation by distance model (Wright, 1965). The island population model suggested by Nevo and Beiles (1989) and confirmed by many studies (e.g., Felsenburg et al., 1991; Nevo et al., 1995; Pagnotta et al., 1995a,b) holds for the Jordanian wild emmer wheat populations.

Spatial-temporal variation. Gene flow in wild emmer wheat and similar populations of predominantly self fertilizing species, is restricted to dispersal by seed (Loveless and Hamrick, 1984; Hamrick and Godt, 1990). Seed dispersal in wild emmer wheat is also restricted (Golenberg, 1989). Felsenburg et al. (1991; and references therein) and Pagnotta et al. (1995b) uncovered a patchy distribution of alleles and genotypes at the micro- and macrogeographic levels; this can be related to environmental heterogeneity which has a potential for generating significant spatial structuring in populations that occupy pronouncedly patchy habitats (Heywood, 1991).

Nevo et al. (1982), Nevo and Payne (1987), and Levy and Feldman (1988) concluded that at least some of the variation in seed storage proteins in wild emmer wheat confers adaptiveness to specific environments. In a country-wide survey, Nevo and Payne (1987) found that gene diversity increases towards the more arid regions. Their statistical analysis indicated correlations between certain seed storage protein phenotypes and environmental attributes such as soil type, altitude, evaporation, annual average temperature, and annual average rainfall. However, in a detailed study at Ammiad, Felsenburg et al. (1991) concluded that the relation between gene diversity and habitat heterogeneity at a microgeographic scale was not clear.

Nevo and Payne (1987) suggested that the geographical patterns of HMW glutenin alleles and their ecological correlates in Israel, may be in part due to natural selection. This suggestion is supported by evidence derived from studies at macro- (Golenberg and Nevo, 1987; Levy and Feldman, 1988) and microgeographic levels (Felsenburg et al., 1991).

In the present and the above-cited studies, clustering of HMW glutenin alleles at macro- and microgeographic levels might have been caused by founder effects or in response to selective pressures, i.e., a particular allele could possess a higher adaptive value in a given environment. A possible effect of selection pressure at a microgeographic level was suggested for the Ammiad site in Israel, where allele *Glu-A1-1a* was confined to a certain transect but totally absent from adjacent transects (Levy and Feldman, 1988; Felsenburg et al., 1991). In the present study, spatial variation varied considerably among the four HMW glutenin loci (Table 1). *Glu-A1-1* varied most widely among locations, with a large range in diversity (0.000 to 0.587), the highest number of pairwise significant differences in gene diversity among populations (44%), and the largest por-

tion of the variance in gene diversity (35.8%; $p < 0.077$). It was followed, in decreasing order, by *Glu-B1-2, Glu-A1-2,* and *Glu-B1-1*. A strong indication of selective pressure can be inferred, for example, from data presented for alleles *Glu-A1-1a, k,* and *o* (Table 1). The first two alleles were classified as rare localized, whereas the third was common localized. All three alleles contributed to the 44% pairwise significant differences among population (*Glu-A1-1t* is a common widespread allele, with no significant differences in frequency among populations). Moreover, the conditional dependency of this locus on the location of the collection site was highly significant (chi-square 47.3, df 33; $p < 0.0509$).

In spite of the possible adaptive role of HMW glutenins, a statistical relationship between their diversity and environmental parameters can appear as a consequence of stochastic processes such as migration or genetic drift, or linkage between the seed storage protein loci and actual loci conferring adaptiveness. In selfing species such as *Triticum* spp. and *Hordeum* spp., however, an additional mechanism is the presence of extensive multilocus associations beyond the physical linkage groups (Gepts, 1990). These correlations provide a working hypothesis (Gepts, 1990; Horovitz and Feldman, 1991) that can be verified experimentally by (1) verifying genotype-habitat associations in different parts of the Fertile Crescent, (2) synthesizing artificial populations containing alternative alleles, establishing these populations in sites with contrasting environments, and monitoring gene frequencies over a number of years, and (3) transplanting genotypes, then follow up their establishment and monitor new allelic and genotypic frequencies.

The year-to-year fluctuations in genotypic frequency averaged over locations did not change significantly for three of the four HMW glutenin loci. The only locus reflecting a temporal variation was *Glu-A1-2* (chi-square 7.56; df 2; $p < 0.022$). The probable cause behind this change could be the presence, on the same site, of alternative genotypes (*Glu-A1-2a*, with a very high frequency, and *Glu-A1-2l*, with a very low frequency) that could have been sampled in different years. The seed pool, as demonstrated at the Ammiad study in Israel (Felsenburg et al., 1991), prevents radical changes in genotype frequency between years due to a founder effect or unusual selection pressure (Levin,1978; Templeton and Levin, 1979) or due to fluctuations in population size or extreme climate. In comparison with spatial factors, and in agreement with theoretical predictions (Karlin, 1979), temporal factors played a very small role in maintaining the polymorphism of HMW glutenins at the local and regional levels.

ALLOZYME SYSTEMS

Polymorphism. Geographical differentiation and ecological associations of allozymic diversity have been demonstrated at a regional scale in studies involving wild emmer wheat populations from Israel and Turkey (Nevo et al., 1991, and references therein). In one of their early reports, Nevo et al. (1982) found that, based on 50 loci, wild emmer wheat had lower mean values of genetic variation than diploid wild barley, however, it displayed distinct local differences over short geographic distances. Genetic index values (*A, P,* and *He*) reported by these researchers imply that populations in the northern part of Israel (mesic environments) were genetically more variable than populations in the south (xeric environments). Moreover, this genetic differentiation is associated with, and predictable in part by climatic regimes and soil type. Striking similarities in the north-to-south gradual decrease in the effective number of alleles, *Ne*, polymorphism, *P-5%*, and gene diversity, *He*, between the Israeli and Jordanian populations can be seen when data in Table 2 are compared with results presented by Nevo and coworkers (e.g., Nevo et al., 1982, 1991, 1995). Predictability of genetic differentiation, although indirect, can be deduced from the highly significant differences among populations in all three parameters, and from the fact that substantial and highly significant portions of variation in all three parameters (57.16–58.9%) were accounted for by differences among locations. The higher levels of polymorphism in the mesic locations may be considered as an adaptive strategy for increasing fitness in the more fluctuating environments (Nevo et al., 1991; and references therein). These results extend the validity of conclusions derived from the Israeli studies to cover a major part of the Fertile Crescent. Moreover, the combined results from Israel and Jordan support the theoretical prediction (Karlin, 1982) that the more heterogeneous environments harbor and maintain higher levels of polymorphism, as compared with less heterogeneous ones.

Some of the wild emmer wheat populations (e.g., Jraineh, Naor, Jarash) may have derived their low *He* and *P-5%* estimates from either founder effects and/or genetic drift (see below). Genetic drift can be a potent force for removing variation, particularly in small populations and when environmental factors push alleles down to low frequencies (Nevo and Beiles, 1989; Foré and Guttman, 1995). Population size may also influence the dispersal potential, because small populations are more likely to be influenced by seed migration. Hence the potential for genetic drift is greater in small populations (Heywood, 1991; Foré and Guttman, 1995).

A close inspection of data on *He, P-5%,* and *Ne* for

the Jordanian populations, as compared with data based on 33 populations from Israel and 4 populations from Turkey, shows that average *He* and *P-5%* for the Israeli and Turkish material were 31.0% and 16.8% lower than parallel estimates for the Jordanian material. On the other hand, *He* for the Jordanian populations was close to the average value reported for 10 central populations from Israel (0.0845 vs. 0.078), and the effective number of alleles in Jordanian populations (*Ne* = 1.064) was close to the number of alleles per locus (*A* = 1.095) found in 7 small-size populations in Israel.

A large portion of the variant alleles were localized (common or rare) (63.6%, as compared to 70.1% in the Israeli-Turkish material, Nevo and Beiles, 1989, and 76% in 12 populations from Israel; Nevo et al., 1982), with significant differences among populations. These figures suggest the presence of considerable differences in allelic content among the 12 populations and are in agreement with earlier findings at regional (Nevo and Beiles, 1989) and local (Nevo et al., 1991) scales.

A comparison of allozyme and HMW-glutenin polymorphism in wild emmer wheat, hordein in wild barley (*Hordeum spontaneum*), and phaseoline in beans (*Phaseolus vulgaris*) reveals that the high levels of seed storage protein diversity contrast with the relatively low levels of allozyme diversity (Hamrick and Godt, 1990). However, the comparisons should be viewed in the light of the fact that (1) analytical methods used for allozymes and seed storage proteins detect different causes of polymorphism, (2) seed storage proteins are encoded by small multigene families that provide additional opportunities for polymorphism (inter- and intragenome associations), and (3) functional constraints acting on seed storage proteins may be lower than those acting on allozymes.

Genetic differentiation among and within populations: Spatial genetic structure is an important factor for strategies of sampling natural populations (Epperson, 1990), either for gene conservation or breeding purposes. The gene flow potential of species has a predominant influence on the partitioning of allozyme variation among populations (Hamrick and Godt, 1990). Direct support for this conclusion can be derived from the close relationship between *Gst* and the species characteristics. Slatkin (1985) and Hamrick (1987) demonstrated that annual selfing species have much lower estimated numbers of migrants per generation and consequently have a smaller portion of their variation partitioned within populations than long-lived species with a potential for long-range gene movement.

Nevo and Beiles (1989) and later Nevo et al. (1991) concluded that wild emmer wheat is characterized by a highly subdivided “archipelago”-type ecological population structure. These researchers found substantially more gene differentiation among and within sometimes geographically very close populations in Israel than between wild emmer in Israel and in Turkey. Partitioning of total gene diversity, based on allozymes in Jordan reinforces this conclusion, however, *Gst* for the Jordanian populations was lower (34.2%) than the value reported by Nevo and Beiles (1989) for 37 wild emmer wheat populations from Israel and Turkey (60.0%). On the other hand, it was much higher than the *Gst* reported for the highly variable population at Ammiad (*Gst* = 12.24%). Nevertheless, when combined with genetic and geographical distances among populations (see below), the patchy genetic structure of wild emmer wheat is revealed once more under the increasing aridity index of Jordan.

Allozyme diversity values calculated for central, peripheral, and marginal populations in Jordan indicate once again (Nevo and Beiles, 1989) that allozyme diversity is generally increases with increasing spatial ecological heterogeneity and unpredictability. From the present findings and other studies of wild emmer wheat, it appears that spatial factors are more important than temporal ones in maintaining allozyme polymorphism in the species, and that aridity is a major differentiating factor (Nevo et al., 1991).

In comparisons with general values determined for annuals, monocots, selfing species, and species dispersed by seed (gravity), (Hamrick and Godt, 1990), Jordanian emmer wheat populations exhibited a lower gene diversity within populations (0.107 vs. 0.200, 0.238, 0.149 and 0.207, respectively). However, the among-population diversity (*Gst*=0.342) was comparable to *Gst* in annuals (0.357), lower than *Gst* in selfing species (0.510), and higher than *Gst* in monocots (0.231) or species dispersed by seed (0.277). An important question is whether this pattern of allozyme variation can be used to predict patterns of genetic variation in other traits.

The pattern observed for the number and percent of monomorphic allozyme loci (Table 2) suggests a major effect of a diversifying balancing selection on allozyme polymorphism (Nevo et al., 1991). On an average, 28.0% of the 36 allozyme loci found 12 populations were monomorphic, with large differences among populations (0.0% in Afana to 53.0% in Jraineh); this average value is much higher than the 14.0% monomorphic loci in 42 gene loci and 37 populations of wild emmer wheat from Israel and Turkey (Nevo and Beiles, 1989). However it is much lower than the 50.0% of the regionally monomorphic loci reported in the same study, or the 37.0% monomorphic loci reported for the Ammiad study (Nevo et al., 1991). The above-cited

results suggest that the likelihood of protected polymorphism increases with increasing heterogeneity of the environment (Karlin, 1982; Brown and Clegg, 1983; Broyles and Wyatt, 1993).

REFERENCES

Allard, R.W., Jain, S.K., and Workman, P.L. 1968. The genetics of inbreeding populations. Adv. Genet. 14: 257–275.

Barrett, S.C.H. and Kohen, J.R. 1991.Genetic and evolutionary consequences of small population size. In: Falk, D.A. and Holsinger, K.E., eds. Genetics and conservation of rare plants. Oxford University Press, Oxford, pp. 3–30.

Brown, A.D.H. and Clegg, M.T. 1983. Isozyme assessment of plant genetic resources. In: Rattazzi, M.C., Scandalios, J.G., and Whitt, G.S., eds. Isozymes: current topics in biological and medical research. Vol. 11. Medical and other applications. Alan R. Liss, New York, pp. 285–295.

Broyles, S.B. and Wyatt, R. 1993. Allozyme diversity and genetic structure in Southern Appalachian populations of poke milkweed, *Asclepias exaltata*. Systematic Botany 18: 18–30.

Ciaffi, M., Lafiandra, D., Porceddu, E., and Benedettelli, S. 1993a. Storage protein variation in wild emmer (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. I. Electrophoretic characterization of genotypes. Theor. Appl. Genet. 86: 474–480.

Ciaffi, M., Lafiandra, D., Porceddu, E., and Benedettelli, S. 1993b. Storage protein variation in wild emmer (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. II. Patterns of allele distribution. Theor. Appl. Genet. 86: 518–525.

Ellstrand, N. and Elam, D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. 24: 217–242.

Epperson, B.K. 1990. Spatial patterns of genetic variation within plant populations. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., and Weir, B.S., eds. Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc. Sunderland, MA, USA, pp. 229–253.

Felsenburg, T., Levy, A.A., Galili, G., and Feldman, M. 1991. Polymorphism of high–molecular–weight glutenins in wild tetraploid wheat: spatial and temporal variation in a native site. Isr. J. Bot. 40: 451–479.

Foré, S.A. and Guttman, S.I. 1995. Spatial and temporal genetic structure of Asclepias verticillata (whoorled milkweed) among prairie patches in a forested landscape. Can. J. Bot. 74: 1289–1297.

Galili, G. and Feldman, M. 1983. Genetic control of endosperm proteins in wheat. I. The use of high resolution one–dimensional gel electrophoresis for the allocation of genes coding for endosperm protein subunits in the common wheat cultivar Chinese Spring. Theor. Appl. Genet. 64: 97–101.

Gepts, P. 1990. Genetic diversity of seed storage proteins in plants. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., and Weir, B.S., eds. Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc. Sunderland, MA, pp. 64–82.

Golenberg, E.M. 1989. Migration patterns and development of multilocus associations in a selfing annual, *Triticum dicoccoides*. Evolution 43: 595–606.

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

Hamrick, J.L. 1987. Gene flow and distribution of genetic variation in plant populations. In: Urbanska, K., ed. Differentiation patterns in higher plants. Academic Press, New York, pp. 53–67.

Hamrick, J.L. and Godt, M.J.W. 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M T., Kahler, A.L., and Weir, B.S., eds. Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, MA, pp. 43–63.

Heywood, J.S. 1991. Spatial analysis of genetic variation in plant populations. Annu. Rev. Ecol. Syst. 22: 335–355.

Horovitz, A. and Feldman, M. 1991. Evaluation of the wild–wheat study at Ammiad. Isr. J. Bot. 40: 501–508.

Jaradat, A.A. 2001. Spatial and temporal genetic structure of wild emmer wheat in Jordan. I. Phenotypic traits. Isr. J. Plant Sci. Suppl. 49: S-53–S-64, this issue.

Jaradat, A.A. and Humeid, B.O. 1990. Morphological variation in wild *Triticum dicoccoides* from Jordan. In: Srivastava, J.P. and Damania, A.B., eds. Wheat genetic resources: meeting diverse needs. Wiley & Sons, Chichester, UK, pp. 215–222.

Karlin, S. 1979. Principles of polymorphisms and epistasis of multilocus systems. Proc. Natl. Acad. Sci. U.S.A. 76: 541–545.

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

Levin, D.A. 1978. Some genetic consequences of being a plant. In: Brussard, P.F., ed. Ecological genetics: the interface. Springer-Verlag, Berlin, pp. 189–212.

Levy, A.A. and Feldman, M. 1988. Ecogeographical distribution of HMW glutenin alleles in populations of the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*. Theor. Appl. Genet. 75: 651–658.

Levy, A.A. and Feldman, M. 1989. Intra- and interpopulation variations in grain protein percentage in wild tetraploid wheat, *Triticum turgidum* var. *dicoccoides*. Euphytica. 42: 251–258.

Levy, A.A., Galili, G., and Feldman, M. 1988. Polymorpphism and genetic control of high molecular weight glutenin subunits in wild tetraploid wheat *Triticum turgidum* var. *dicoccoides*. Heredity 61: 63–72.

Lewis, A.A. and Zaykin, D. 1999. Genetic data analysis: Computer program for the analysis of allelic data. Version 1.2 {d2I}. http://chee.unm.edu/gda/

Loveless, M.D. and Hamrick, J.L. 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15: 65–95.

Marshall, D.R. and Allard, R.A. 1970. Isozyme polymorphisms in natural populations of *Avena fatua* and *Avena*

barbata. Heredity 25: 373–382.

Marshal, D.R. and Brown, A.H.D. 1975. Optimum sampling strategies in genetic conservation. In: Frankel, O.H. and Hawkes, J.G., eds. Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, pp. 53–70.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. 70: 3321–3323.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press. New York.

Nei, M. and Chesser, R.K. 1983. Estimation of fixation indices and gene diversities. Ann. Hum. Genet. 47: 253–259.

Nevo, E. 1983. Adaptive significance of protein variation. In: Oxford, G.S. and Rollinson, D., eds. Protein polymorphism: adaptive and taxonomic significance. Academic Press, London. pp. 239–282.

Nevo, E. 1987. Plant genetic resources: prediction by isozyme markers and ecology. In: Rattazi, M.C., Scandalios, J.G., and White, G.S., eds. Isozymes: current topics in biological and medical research. Vol. 16. Agriculture, physiology and medicine. Alan R. Liss, New York. pp. 247–267.

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

Nevo, E. and Payne, P.I. 1987. Wheat storage proteins: diversity of HMW glutenin subunits in wild emmer wheat from Israel. 1. Geographical patterns and ecological predictability. Theor. Appl. Genet. 74: 827–836.

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

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

Nevo, E., Beiles, A., Gutterman, Y., Storch, N., and Kaplan, D. 1984. Genetic resources of wild cereals in Israel and vicinity. I. Phenotypic variation within and between populations of wild wheat, *Triticum dicoccoides*. Euphytica 33: 717–735.

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

Nevo, E., Beiles, A., and 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.

Nevo, E., Noy-Meir, I., Beiles, A., Krugman, T., and Agami, M. 1991. Natural selection of allozyme polymorphisms: micro–geographical spatial and temporal ecological differentiations in wild emmer wheat. Isr. J. Bot. 40: 419–449.

Nevo, E., Pagnotta, M.A., Beiles, A., and Porceddu, E. 1995. Wheat storage proteins: glutenin diversity in wild emmer wheat, *Triticum dicoccoides*, in Israel and Turkey. 3. Environmental correlates and allozymic associations. Theor. Appl. Genet. 91: 415–420.

Pagnotta, M.A., Nevo, E., Beiles, A., and Porceddu, A. 1995a. Wheat storage proteins: glutenin diversity in wild emmer, *Triticum dicoccoides*, in Israel and Turkey. 2. DNA diversity detected by PCR. Theor. Appl. Genet. 91: 409–414.

Pagnotta, M.A., Nevo, E., Beiles, A., and Porceddu, A. 1995b. Wheat storage proteins: glutenin diversity in wild emmer, *Triticum dicoccoides*, in Israel and Turkey. 3. Environmental correlates and allozymic association. Theor. Appl. Genet. 91: 415–420.

Palmer, M.W. and Maurer, T.A. 1997. Does diversity beget diversity? A case study of crops and weeds. J. Veget. Sci. 8: 235–240.

Roger, A.R. and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9: 552–569.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39: 53–65.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry. W.H. Freeman and Company, New York.

StatSoft. 1998. STATISTICA for Windows. Vol. I & II. 2nd Ed. Tulsa, OK.

Templeton, A.R. and Levin, D.A. 1979. Evolutionary consequences of seed pools. Am. Natur. 114: 232–249.

Weir, B.S. 1996. Genetic data analysis II. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Whitkus, R. 1985. FORTRAN program for computing genetic statistics from allelic frequency data. J. Heredity 76: 142.

Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution 46: 608–615.

Wright, S. 1965. The interpretation of population structure by F–statistics with special regard to systems of mating. Evolution 19: 395–420.

Zang, Q. and Allard, R.W. 1986. Sampling variance of the genetic diversity index. J. Hered. 77: 54–55.

Zar, J.H. 1996. Biostatistical analysis. 3rd ed. Prentice Hall, Upper Saddle River, NJ.