

PHENOTYPIC AND ALLOZYME VARIATION IN MEDITERRANEAN AND DESERT POPULATIONS OF WILD BARLEY, *HORDEUM SPONTANEUM* KOCH

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Abstract.—Populations of wild barley, *Hordeum spontaneum* Koch, were collected in two distinct climatic regions, desert and Mediterranean. Plants from five desert and five Mediterranean populations were compared and contrasted for extent and structure of phenotypic variation. These same 10 and one other population from each region were analyzed for allozyme variation. In a field trial of phenotypic diversity, two phenological and 14 morphological traits were examined. Study of allozyme variation was performed using eight enzyme systems encoding for 13 loci. Plants from the desert and Mediterranean regions were significantly different in seven of 16 phenotypic traits, exhibited a high (30%) interregional component of phenotypic variation, and showed a high degree of segregation on a principal component scattergram indicating ecotypic differentiation. Mediterranean populations were twice as variable as desert populations in reproductive growth parameters (stem and spike length) and grain filling (spikelet weight), but half as variable for onset of reproduction. The extent and structure of phenotypic and allozyme variation did not match. The Mediterranean and desert populations did not differ in amount of allozyme variation as estimated by mean number of alleles per locus, effective number of alleles, polymorphism, and gene diversity (n_a , n_e , P , and H_e), did not segregate on the basis of population genetic distances, and exhibited a low proportion of interregion allozyme diversity (2%). No effect of selection on allozyme distribution was detected. Our results suggest that the adaptation of plants originating from desert and Mediterranean environments is reflected in phenotypic but not in allozyme variation.

Key words.—Allozymes, desert, Mediterranean, phenotypic traits, variation, wild barley.

Received August 3, 2001. Accepted April 8, 2002.

Phenotypic and genetic differentiation between populations of a species occupying an array of environments was first experimentally demonstrated by Turesson (1922), and plant ecotypes along broad environmental gradients have been detected in many studies (e.g., Ehleringer and Clark 1988; Grant and Wilken 1988; Macdonald and Chinnappa 1988, 1989). Such ecotypes are assumed to be locally adaptive, and several rigorous tests of this assumption have been conducted (Waser and Price 1985; Jordan 1992; Bennington and McGraw 1996; Nagy and Rice 1997; Stanton and Galen 1997).

A number of studies have addressed the question of differences in the population genetic pattern in xeric versus mesic environments (Clegg and Allard 1972; Hamrick and Allard 1972, 1975; Nevo and Beiles 1988; Comes and Abbott 1999). Higher levels of population subdivision were expected in xeric environments either because of their high temporal and spatial heterogeneity, which negatively influences dispersal of genes among populations and makes them susceptible to local extinction and recolonization (Comes and Abbott 1999), or because of microhabitat selection (Nevo and Beiles 1988). However, if gene flow is sufficiently large and loci are selectively neutral, no difference in population genetic structure between mesic and xeric regions is expected under models in which exchange of genes is a function of geographic distance (isolation by distance and stepping stone models). The latter can be tested by measuring the relationship between gene flow and geographic distance among populations. However, if such a relationship is not found, any inferences about the effect of selection on population genetic structure without an explicit testing of local adaptation are

anecdotal. In neither of the above studies of genetic variation in xeric versus mesic environments was a direct test undertaken to examine the effects of selection. Only in a few studies of natural plant populations have estimates of both the effect of habitat-specific selection and gene flow been obtained (McGraw and Antonovics 1983; Waser and Price 1985; Williams and Waser 1999). Population genetic analysis combined with an explicit test of local adaptation appears to be a useful approach to studying xeric versus mesic environments for which different selection effects on population genetic structure have been suggested but rarely tested (Clegg and Allard 1972; Hamrick and Allard 1972; Nevo et al. 1979; Nevo and Beiles 1988).

Demographic and stochastic genetic factors, such as population size and density, isolation, gene flow, and genetic drift may influence the process of genetic differentiation among populations. Isolation, small population size, and genetic drift are expected to reinforce genetic differentiation among populations (Nei et al. 1975; Cohan 1984; Ellstrand and Elam 1993; Epperson 1995), while increased gene flow should retard it (Mayr 1963; Loveless and Hamrick 1984; Heywood 1991). The distribution of genetic variation at different levels of population subdivision (population genetic structure) and the extent of such variation depend on factors affecting all loci similarly (genetic drift and mating system) as well as on forces having differential effects on loci (natural selection and mode of inheritance). Therefore, a comparison of genetic structure for allozymes versus quantitative traits allows valuable insight into the relative importance of both stochastic and deterministic forces at two levels of gene expression (proteins vs. the whole plant), and the modes of

inheritance (single gene vs. polygenic). Such a comparison is especially fruitful when local adaptation is directly detected by reciprocal introductions.

Although some studies have suggested possible selection effect on allozymes (Heywood and Levin 1985; Lack and Kay 1988; Lönn 1993; Lönn et al. 1996; Van Rossum et al. 1997), in most studies allozymes have been assumed to be selectively neutral. Both molecular markers and quantitative characters can be either neutral or experience differential selection pressure depending on how tightly they are related to fitness. A quantitative trait, despite its polygenic nature, was shown to contain exactly as much information about population relationships as one single-locus marker (Rogers and Harpending 1983). This means that if both the polygenic phenotypic traits and single-locus allozymes are neutral they will show a similar extent of variation and similar patterns of population structure across populations and loci. Moreover, if phenotypic traits and allozymes are selectively neutral, their patterns of variation do not have to be related to environmental differences, that is, no differences between individual plants and populations from mesic and xeric environments are expected. However, if both regions are clearly differentiated in either extent or structure of variation in particular loci and adaptation is detected by reciprocal introduction, a region-specific selection effect on these loci cannot be ruled out.

Multilocus associations are predicted to occur more often in predominantly self-pollinating than in outcrossing species (Brown 1979). Consequently, an association between enzyme loci and loci determined by or linked to other traits is expected to be higher in selfing than in outcrossing species. Indeed, similar population differentiation revealed by enzyme loci and morphological traits, suggesting an association between these two classes of loci, was reported for predominantly inbreeding *Veronica peregrina* (Keeler 1978) and *Carex lepidocarpa* (Hedren and Prentice 1996), whereas no such association was observed in outcrossing *Phlox drummondii* (Levin 1977; Schwaegerle et al. 1986), *Pseudotsuga menziesii* (El-Kassaby 1982), *Ipomopsis aggregata* (Wolf and Campbell 1995), and *Senecio gallicus* (Comes and Abbott 1998, 2000). In a study of Price et al. (1984) associations between estimates of population differentiation were significant in the three selfing species (*Avena barbata*, *Hordeum jubatum*, and *Hordeum vulgare*) but not in the predominantly outcrossing *Clarkia williamsonii*. Only in a few studies, however, were allozyme and quantitative trait variation not only compared with each other but also related to distinct environmental heterogeneity to infer the effect of selection on them. Similar population differentiation by allozymes and phenotypic traits reflecting distribution of two types of environments was observed in the predominantly inbreeding *A. barbata* (Hamrick and Allard 1972, 1975). However, in a study of the outcrossing *Clarkia dudleyana* (Podolsky and Holtsford 1995) a discordant population structure was found when comparing allozyme, discrete, and continuous morphological trait variation with only discrete morphological traits indicating selection effect.

Self-pollination is a life-history character limiting gene flow (as compared with outcrossing) and enhancing local differentiation (Loveless and Hamrick 1984; Heywood 1991;

Linhart and Grant 1996). This character is associated with high estimates of F_{ST} (Hamrick and Godt 1989), but it remains unclear how much (if any) local selection contributes to the population differentiation in selfers and how the effect of selection is reflected at two levels of genetic expression (single-locus enzymes and polygenic quantitative traits).

In our study of the predominantly self-pollinating *Hordeum spontaneum* we analyzed population structure for both allozyme and quantitative traits in mesic Mediterranean and xeric desert environments of Israel. A sharp gradient of increasing aridity and rainfall unpredictability separates the desert and Mediterranean regions in Israel. Wild barley, *H. spontaneum*, is one of the dominant annual components of open park forests and Mediterranean grassland, where it thrives. However, it also penetrates into the less favorable Judean and Negev Deserts (Harlan and Zohary 1966), where it occupies accumulating runoff ephemeral river valleys (wadies) and exhibits high patchiness and isolation. The abundance of *H. spontaneum* decreases sharply from Mediterranean to desert environments, but population density within the wadies can be as high as in mesic populations (S. Volis, pers. obs.). Genetic diversity of *H. spontaneum* was studied across its distributional range in a number of parameters including morphological traits and isozyme and DNA markers (Brown et al. 1978; Snow and Brody 1984; Nevo et al. 1986c, 1998; Saghai Maroof and Allard 1990; Chalmers et al. 1992; Pakniyat et al. 1997; Volis et al. 2001). However, no satisfactory attempt was made to disentangle stochastic and deterministic causes of this species variability. The latter requires local adaptation to be tested and demonstrated. Our study of genetic variability in barley populations differs from those previously done in that it was flanked by a rigorous test of local plant adaptation (Volis et al. 2002). Therefore, patterns of allozyme and quantitative trait variation are not simply compared but are also analyzed for a possible contribution of natural selection to the observed genetic variation.

MATERIALS AND METHODS

Experimental Methods

Phenotypic traits

Approximately 50 plants/population of wild barley were collected in 1992 and 1994 from five populations from the Mediterranean and five populations from the desert regions in Israel. Plants from each population were collected along a transect(s) (the number of transects was a function of population geography) with the collected plants spaced 2–3 m apart. One spike per plant was harvested and separately bagged. Key geographic and climatic data on each population is presented in Table 1. Meteorological data were taken from multiple-year records of the Meteorological Service of Israel. Five Israeli populations were from Shefela and Judean Hills (Mediterranean), and five populations were from the Negev and Judean Deserts (desert).

Seeds of *H. spontaneum* were sown in a greenhouse and the three-week-old plantlets were transplanted into an open field at Ben Gurion University, Beer Sheva. Initially the field trial was designed to test for differences in phenotypic plasticity between core and peripheral plants. A randomized block

TABLE 1. Ecogeographical data for populations of *Hordeum spontaneum*. Rn, annual rainfall (mm); Tm, annual temperature; Ta, mean temperature in August; Tj, mean temperature in January.

Station	Geographical location	Region	Altitude (m)	Climatic parameters			
				Rn	Tm	Ta	Tj
Yavne*	Mediterranean coast	Mediterranean	10	529	20	26	13
Bet Shemesh	Judean Hills	Mediterranean	350	482	17	26	10
Jerusalem	Judean Hills	Mediterranean	810	537	18	24	9
Shoresh	Judean Hills	Mediterranean	680	599	19	26	10
Bet Guvrin	Shefla Hills	Mediterranean	270	408	19	26	11
Kyriat Gat	Shefla Hills	Mediterranean	130	382	19	26	11
Jeriho	Judean Desert	desert	-260	162	23	30	14
Dead Sea	Judean Desert	desert	-300	67	23	32	14
Yeruham	Negev Desert	desert	380	104	19	26	10
Ein Yarkeam	Negev Desert	desert	430	121	19	26	10
Sede Boqer	Negev Desert	desert	470	90	19	26	9
Dimona*	Negev Desert	desert	400	144	19	26	11

* Used only for allozyme genetic analysis.

design was established with each of three blocks consisting of three rows, one each of three water irrigation treatments that were the equivalent of 50, 100, and 150 mm rainfall over the experiment. The actual water amount available to each plant, supplementary irrigation plus rainfall, equaled 250, 300, and 350 mm rainfall. Each row was 1.5 m wide and contained a single drip line running down its center containing 2-L/hour drippers spaced 0.5 m apart. For each population, four seedlings were transplanted one after the other in each row. Irrigation and fertilization were conducted once a week during the growing season through the drip lines (an equal amount of water was given each week during the growing season). After the appearance of reproductive tillers, the first three reproductive tillers of each plant were tagged with colored tags representing the first, second and third tiller in order of awn appearance. Each tiller was measured for culm length, flag and penultimate leaf length, spike length, awn length, number of nodes, internode length, and total tiller height, and the number of days to anthesis and awning were recorded. At senescence all spikes were harvested and the number of spikelets per spike and the average spikelet and seed weight were measured.

Electrophoresis

Starch gel electrophoresis was performed on 12 populations of wild barley (the same 10 as for phenotypic variation plus one more per region collected in 1990; Table 1). Eight enzyme systems encoding for 13 loci were examined following protocol and recipes of Brody and Mendlinger (1980) and Mendlinger and Zohary (1995). Population sample size was 20 plants.

One seed from each selected spike was germinated and grown in a greenhouse. Leaves from two-week-old seedlings were cut and macerated in two drops of distilled water and a drop of bromophenol blue. The extract was absorbed on filter paper wicks and placed into a gel. The starch gel was prepared by boiling 39 g of hydrolyzed potato starch (S-4501, Sigma) dissolved in 300 ml gel buffer with subsequent degassing by a vacuum pump. Three buffer systems, Tris-citrate (TC), Tris-malate (TM), and a Poulik discontinuous buffer (Poulik) were used (Mendlinger and Zohary 1995).

Samples from 15 different plants, five each from three

populations, plus a control (a commercial cultivar of cultivated barley) were placed in each gel. Gels were run in a refrigerator at 4°C on a constant current of 100 mA and a voltage of 100 (TM), 110 (TC), or 200 (Poulik) for 3–4 h. After electrophoresis, each gel was sectioned into three slices with each slice stained for a different enzyme. The eight enzymes examined, the abbreviations employed, EC numbers, number of loci in each enzyme system, and the buffer used are: (1) catalase, *Cat*, EC 1.11.1.6, one locus, Poulik buffer; (2) general protein, *Gp*, EC 4.1.1.39, one locus, TM buffer; (3) glutamate dehydrogenase, *Gdh*, EC 1.4.1.2, one locus, TC buffer; (4) esterase, *Est*, EC 3.1.1.2, three loci, TC buffer; (5) malate dehydrogenase, *Mdh*, EC 1.1.1.37, two loci, TM and TC buffer; (6) phosphoglucomutase, *Pgm*, EC 2.7.5.1, one locus, TM buffer; (7) phosphoglucose isomerase, *Pgi*, EC 5.3.1.9, two loci, TM buffer; and (8) 6-phosphogluconate dehydrogenase, *6-Pgd*, EC 1.1.1.44, two loci, TM buffer. After staining the gels were scored and then fixed in 10% glacial acetic acid for 24 h and stored at 4°C. Alleles were scored according to their rate of movement from the origin.

Statistical Analysis

Phenotypic traits

Comparison of phenotypic traits and trait complexes.—The analysis of phenotypic variation in plants of desert and Mediterranean origin included: comparison of phenotypic plasticity, comparison of traits and trait complexes in two regions, and assessment of the extent and structure of variability at two hierarchical levels (among and within regions).

Phenotypic plasticity in plants from the two regions was compared by two-way ANOVA with regions and water treatments as factors. A significant regions × water treatment interaction would indicate a different plastic response to water treatment between Mediterranean and desert plants.

Phenotypic differences between Mediterranean and desert plants were analyzed by nested ANOVA with two fixed effects (water treatment and region) and one random effect (populations nested within regions). The nested ANOVA was run for each trait separately.

The overall phenotypic differences between plants from

two regions were analyzed by principal components analyses (PCA). The analysis was run without distinction between water treatments using the same 16 morphological and phenological traits.

Assessment of phenotypic variability.—To estimate the extent of variation in phenotypic traits, we followed the main idea of Levene's test of sample variance equality (Sall and Lehman 1996), that is, use of the absolute values of residuals instead of original values. A one-way ANOVA with water treatments as the factor removed the water effect (we found the water treatment \times region interaction to be insignificant for all traits). The residuals from this ANOVA were used in nested ANOVA and MANOVA with regions as a fixed effect and populations nested within regions as a random effect. Thus, comparison of the absolute values of residuals done by ANOVA for each trait allowed us to estimate the extent of variation in the trait per se when a significantly larger regional value denotes higher trait variability. The overall differences in extent of the traits' variability were analyzed by nested MANOVA and Wilcoxon's signed-ranks test. MANOVA was used to test whether the extent of overall trait variability differed between the two regions. However, MANOVA cannot test the general consistency of the differences in variability across the set of traits. This can be detected by Wilcoxon's signed-ranks test if one of the regions has consistently higher variation in most of the traits.

Structure of variation in phenotypic traits was analyzed using original trait values. This was done after running nested ANOVA on original trait values by partitioning the total variance between regions, among populations within a region and among individuals within a population. A mixed model was used with one fixed effect (water treatment) and two random effects (regions and populations nested within regions). Regions were considered a random effect to include the regional level into hierarchical variance partitioning. In the same way the variance was partitioned into among- and within-population components for each region separately.

All the statistical analyses were done using JMP version 3.1.2 statistical package (Sall and Lehman 1996).

Allozymes

All analyses of genetic data were performed using POPGENE version 1.31 (Yeh et al. 1998). The amount of genetic variation for each population was estimated by calculating the mean number of alleles per locus, n_a ; the effective number of alleles, n_e ; the proportion of polymorphic loci, P ; and the expected percent heterozygosity (i.e., the extent of genetic diversity), H_e . Bootstrapping with random reassignment to regenerate the original sample sizes repeated 1000 times was applied for estimating 95% confidence intervals over alleles and loci. This was done by the program Resampling Stats (Simon 1995). The partitioning of genetic diversity into its three components, within populations, among populations within a region, and between the two regions, was accomplished by using Nei's G_{ST} -statistics (Nei 1973). The Ewens-Watterson test for neutrality (1000 permutations per test; Manly 1985) was performed for each locus to detect possible effects of selection on interpopulation allele distribution.

Pairwise estimates of F_{ST} for each pair of populations were

used to construct regional and overall scatterplots of F_{ST} on geographic distance of separation, and the relationship between F_{ST} and geographic distance were subjected to a Mantel test (Mantel 1967). Nei's unbiased measures of genetic distance (Nei 1978) were calculated and used in a cluster analysis (unweighted pair groups means method, UPGMA; SIMGEND and SAHN of NTSYSpc, Rohlf 1998). Genetic and geographic distances were also subjected to a Mantel test. Evaluation of the significance of each matrix correlation employed a comparison of observed Mantel test statistic, Z , with its random distribution generated by 1000 random permutations (using MXCOMP of NTSYSpc).

RESULTS

Phenotypic Variation

Phenotypic traits associated with Mediterranean and desert environments

Water treatment had a significant effect on plant growth (internode length, total plant height, and culm height), phenology (days to awn appearance and anthesis), spikelet weight, and ratio seed/spikelet weight (Table 2). There was no significant region \times water interaction for any trait, indicating no difference in phenotypic plasticity of plants of different origin (Mediterranean versus desert).

Plants from the two regions (Mediterranean and desert) differed in seven of 16 traits: both phenological traits (days to awn appearance and to anthesis), spikelet and seed mass, awn and spike length, and number of nodes (Table 2). Plants from the mesic environment had longer spikelets and awns and heavier spikelets and seeds than plants from the xeric environment. The bolting stage and anthesis were delayed in the Mediterranean ecotype relative to that of the desert plants, and the frequency distribution of the latter ecotype was skewed (Fig. 1).

PCA was performed to visualize the overall phenotypic differences between the regions. The first three principal components explained 71.8% of the total variance. Stem and spike lengths, number of nodes, and weight of seed contributed the most to PC1; internode length and length of first two leaves to PC2; and number of spikelets in a spike, onset of anthesis/awning, and spikelet weight to PC3. The differences between regional ecotypes reflected in their position on a scattergram were consistent under all three water treatments (data not shown) and when the water treatments were pooled (Fig. 2).

Extent and structure of phenotypic variation

There was no difference in the extent of variation over all traits as tested by multivariate analysis of variance (MANOVA) and Wilcoxon's signed-rank test conducted on absolute values of residuals (Table 3). The extent of variation had opposite patterns for different traits in plants of Mediterranean and desert origin. Mediterranean plants were more variable in spikelet weight and length parameters of stem and spike but less variable in start of reproduction than plants from desert populations (Table 3).

The structure of phenotypic variation analyzed by the partitioning of variation in original traits among three levels

TABLE 2. Phenotypic differences between Mediterranean and desert plants analyzed by nested ANOVA with two fixed effects (water treatment and region) and one random effect (populations nested within regions). Interaction treatment \times region was not significant for all traits. Lengths are in centimeters and weights are in milligrams. Differences between regions are shown for traits with significant region effect.

Trait	Source of variation			
	Treatment	Region	Mediterranean vs. desert	Population (region)
Phenological				
Days to awn appearance	5.2**	12.6**	†	9.3***
Days to anthesis	5.0**	12.9**	†	8.8***
Morphological				
Total height	11.2***	5.0		15.6***
Spike length	2.3	9.4*	†	17.4***
Awn length	1.9	8.2*	†	17.6***
Culm length	11.5***	3.7		13.9***
Number of nodes	2.5	7.8*	†	16.2***
Internode length	11.3***	1.7		2.8**
Flag leaf length	0.9	3.9		15.8***
Penultimate leaf length	0.2	2.8		16.6***
Flag leaf width	0.3	0.3		17.7***
Penultimate leaf width	0.8	3.4		21.8***
Number of spikelets in a spike	0.8	2.6		6.7***
Spikelet weight	8.7***	19.0***	†	9.3***
Seed weight	0.5	8.9*	†	22.5***
Ratio seed/spikelet weight	6.6**	0.4		11.3***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Significantly larger regional mean (Mediterranean vs. desert).

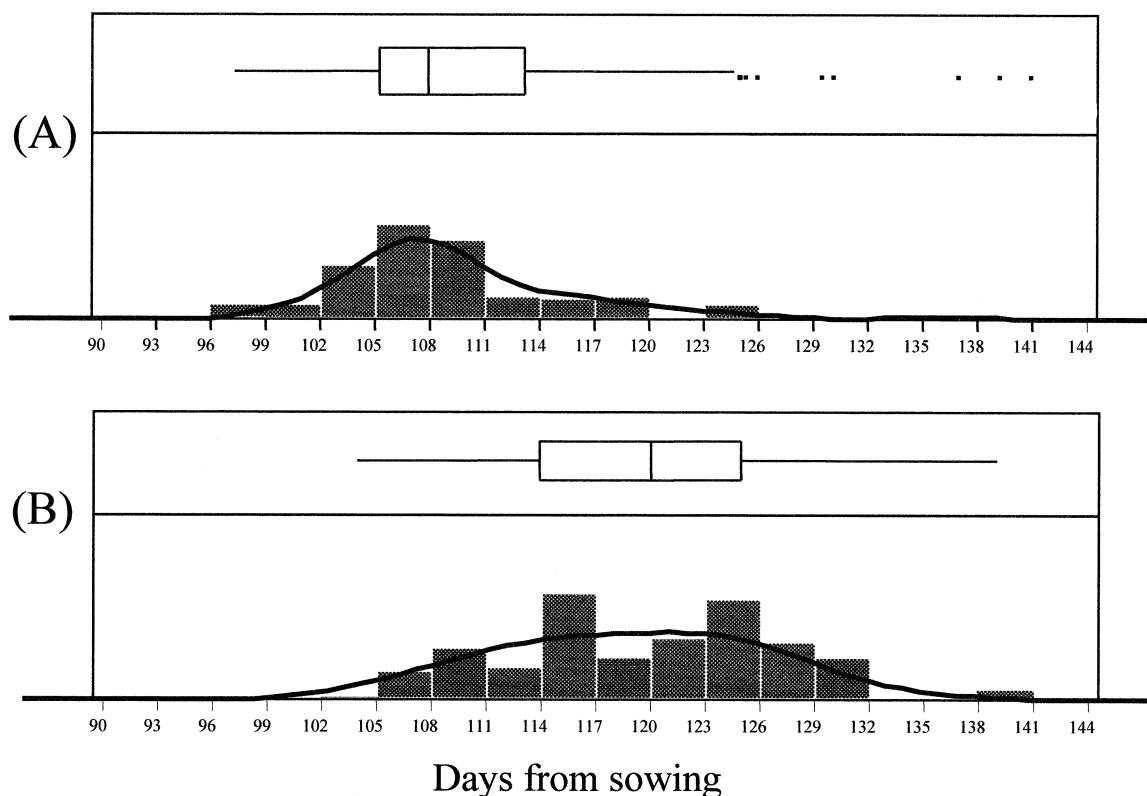


FIG. 1. Frequency distribution and the outlier box plots for the number of days to anthesis in the desert (A) and Mediterranean (B) plants. The box shows 50% (median) and the range of the 25% and 75% quartiles. The whiskers extend to the farthest point that is still within 1.5 interquartile ranges from the quartiles, and the points farther away are outliers

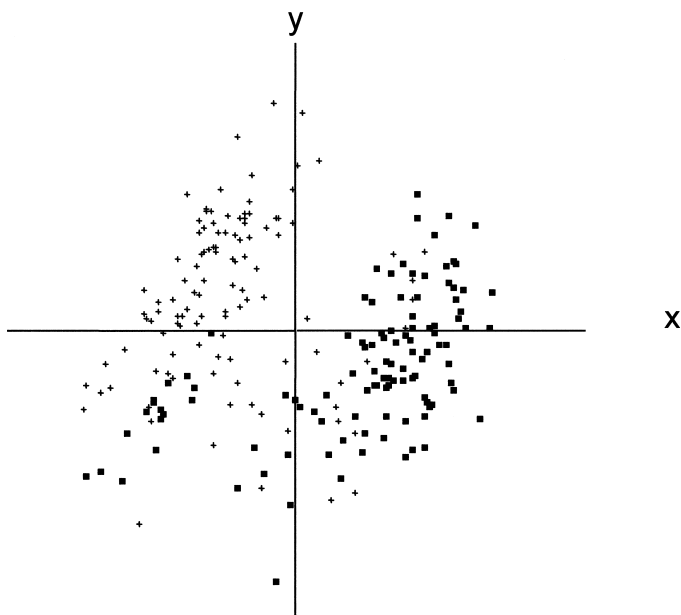


FIG. 2. Principal component analysis of 16 phenotypic traits in 10 populations of wild barley from Mediterranean (boxes) and desert (crosses) regions. Axes are the first two principal components; each dot represents a single plant. Three water treatments are pooled.

(within populations, among populations within a region, and between regions) revealed a high percentage of variation at the regional level (30 ± 4 on average; Table 4). The mean (over all traits \pm SE) percentage of variation between regions exceeded the variation among populations within a region (24 ± 3) and approached the variation within populations (46 ± 4). In seven of 16 traits the interregional variation exceeded the other two components of variation (days to anthesis and to awn appearance; length of spike, awn, flag, and penultimate leaf; spikelet and seed weight). Intrapopulation variation on average was higher in the Mediterranean as compared with the desert region (38 ± 3 and 29 ± 5 , respectively), but in four of 16 traits (flag and penultimate leaf length, seed weight, and ratio seed/spikelet) desert populations were more differentiated than Mediterranean ones.

Allozyme Variation

Extent of allozyme variation within populations

No significant differences were found for within-population genetic diversity in desert versus Mediterranean populations for mean number of alleles per locus (range = 1.15–1.69, mean = 1.37; range = 1.31–1.85, mean = 1.50, respectively), effective number of alleles (range = 1.04–1.20, mean = 1.12; range = 1.03–1.28, mean = 1.16), proportion of loci polymorphic (range = 0.08–0.46, mean = 0.30; range = 0.23–0.62, mean = 0.36), and gene diversity (range = 0.026–0.121, mean = 0.085; range = 0.028–0.167, mean = 0.110). There were also no differences between regions in n_a , n_e , P , and H_e calculated for two gene pools: desert and Mediterranean (Table 5).

Structure of allozyme variation

No significant differences were found in total genetic diversity (H_T) of the two regions (desert and Mediterranean; 0.094 vs. 0.129), within (H_S) or between (D_{ST}) population components (0.077 vs. 0.099 and 0.017 vs. 0.030, respectively; Table 6). The percentage of among-population variation within a region (G_{ST}) was 12.8% (95% CI = 0.070–0.170) for the desert and 12.5% (95% CI = 0.050–0.205) for the Mediterranean.

The partitioning of the total genetic diversity (H_T) into three components: intrapopulation (H_C), interpopulation (D_{CS}), and interregion (D_{ST}) revealed that the proportion of variation among populations within a region was 12%, variation within populations was 86%, and between regions was only 2% (Table 6). The low proportion of variation due to interregional differences was consistent over all loci (range = 0.004–0.066). Cluster analysis performed on pairwise genetic distances among populations revealed no population differentiation due to their regional position (Fig. 3).

Large genetic differences between populations irrespective of their regional position may suggest: (1) varying degree of isolation of populations from each other and concomitant effect of genetic drift; or (2) local effect of natural selection. The Ewens-Watterson test for neutrality detected no evidence of selection for allele distribution (Table 7). The homozygosity F -value generated by permutation test was much larger for all loci than that expected from a given distribution for $\alpha = 5\%$ level (Manly 1985). The approximate probability of obtaining the observed or lower homozygosity F by chance (as indicated by corresponding percentage points in Table 7) ranged between 50% and 90%.

Mantel tests performed on pairwise genetic versus geographic distance and F_{ST} versus geographic distance revealed no significant correlation ($r = 0.05$, $P > 0.05$; $r = 0.14$, $P > 0.05$, respectively). A scatterplot of F_{ST} on geographic distance did not show any change in the degree of scatter with geographic distance (Fig. 4). There was also no significant relationship of pairwise F_{ST} with geographic distance in either region ($r = 0.30$, $P > 0.05$; $r = -0.17$, $P > 0.05$ in Mediterranean and desert regions, respectively).

DISCUSSION

Plants from the two regions, Mediterranean and desert, were significantly different in a number of phenotypic traits and segregated on the scattergram based on the first two principal components. In addition, there was a high (30%) interregional component for phenotypic variation. Because many phenotypic traits are related to fitness and are under selection pressure, at least part of the differences between Mediterranean and desert populations appears to be adaptive and to indicate regional ecotypic differentiation. Snow and Brody (1984) detected different ecotypes of *H. spontaneum* in Israel, and our study of genotype \times environment interactions with reciprocal transplanting of desert and Mediterranean genotypes into native and alien environments supports local adaptation (Volis et al. 2002). Early reproduction in the desert ecotype was clearly advantageous at the desert location as revealed by reciprocal transplanting of seedlings. The colonizing success of indigenous versus alien genotypes a year

TABLE 3. Extent of variation in phenotypic traits analyzed by nested ANOVA, nested MANOVA, and Wilcoxon's signed-rank test. Analyses were run on absolute values of residuals on water treatments. Factors: regions and populations (nested within regions).

Traits	Source of variation	df	MS	F	Difference Mediterranean vs. desert
Phenological					
Days to awn appearance	regions	1	4088	22.6**	<
	populations (regions)	8	182	6.3***	
	error	219	29		
Days to anthesis	regions	1	3818	23.3**	<
	populations (regions)	8	165	6.5***	
	error	219	25		
Morphological					
Total height	regions	1	6241	15.8**	>
	populations (regions)	8	397	4.4***	
	error	229	90		
Spike length	regions	1	191.9	11.7**	>
	populations (regions)	8	16.5	5.6***	
	error	220	2.9		
Awn length	regions	1	35.1	2.8	=
	populations (regions)	8	12.6	8.4***	
	error	220	1.5		
Culm length	regions	1	3849	13.2**	>
	populations (regions)	8	290	4.0***	
	error	211	73		
Number of nodes	regions	1	0.76	3.8	=
	populations (regions)	8	0.20	1.6	
	error	223	0.13		
Internode length	regions	1	10.6	1.4	=
	populations (regions)	8	7.2	2.7**	
	error	220	2.6		
Flag leaf length	regions	1	300.3	4.7	=
	populations (regions)	8	64.1	9.7***	
	error	220	6.6		
Penultimate leaf length	regions	1	354	3.4	=
	populations (regions)	8	102	12.7***	
	error	220	8.1		
Flag leaf width	regions	1	0.13	1.5	=
	populations (regions)	8	0.08	4.0***	
	error	220	0.02		
Penultimate leaf width	regions	1	0.19	0.6	=
	populations (regions)	8	0.30	12.0***	
	error	220	0.02		
Number of spikelets in a spike	regions	1	0	0	=
	populations (regions)	8	34.4	9.0***	
	error	220	3.8		
Spikelet weight	regions	1	5.6×10^{-3}	10.1*	>
	populations (regions)	8	5.5×10^{-4}	9.7***	
	error	211	5.7×10^{-5}		
Seed weight	regions	1	3×10^{-4}	0.4	=
	populations (regions)	8	9×10^{-5}	9.6***	
	error	213	9×10^{-6}		
Ratio seed/spikelet weight	regions	1	2.1×10^{-2}	2.1	=
	populations (regions)	8	9.9×10^{-3}	5.4***	
	error	216	1.8×10^{-3}		
MANOVA			Wilk's Lambda	F	
	regions		0.99	0.01	
	populations (regions)		0.90	2.87**	
Wilcoxon's signed-ranks test (n = 16)			test statistic = 2		

* P < 0.05, ** P < 0.01, *** P < 0.001.

TABLE 4. Phenotypic traits' variance components (%) partitioned between regions, among populations within a region, and within populations. σ_w^2 , within populations; σ_{bp}^2 , among populations; and σ_{br}^2 , between regions.

Trait	Desert		Mediterranean		Both regions		
	σ_w^2	σ_{bp}^2	σ_w^2	σ_{bp}^2	σ_w^2	σ_{bp}^2	σ_{br}^2
Phenological							
Days to awn appearance	0.76	0.24	0.55	0.45	0.37	0.24	0.40
Days to anthesis	0.77	0.23	0.57	0.43	0.38	0.21	0.39
Morphological							
Total height	0.96	0.04	0.50	0.50	0.47	0.35	0.18
Spike length	0.70	0.30	0.51	0.49	0.34	0.27	0.39
Awn length	0.54	0.46	0.57	0.43	0.30	0.14	0.56
Culm length	1.00	0.00	0.53	0.47	0.53	0.35	0.12
Number of nodes	0.89	0.11	0.48	0.52	0.36	0.34	0.30
Internode length	0.92	0.08	0.94	0.06	0.93	0.03	0.04
Flag leaf length	0.46	0.54	0.72	0.28	0.38	0.20	0.42
Penultimate leaf length	0.40	0.60	0.82	0.18	0.40	0.20	0.41
Flag leaf width	0.64	0.36	0.55	0.45	0.60	0.40	0.00
Penultimate leaf width	0.60	0.40	0.51	0.49	0.41	0.37	0.22
Number of spikelets in a spike	0.82	0.18	0.75	0.25	0.72	0.15	0.13
Spikelet weight	0.82	0.18	0.62	0.38	0.30	0.25	0.44
Seed weight	0.39	0.61	0.54	0.46	0.24	0.18	0.58
Ratio seed/spikelet weight	0.62	0.38	0.74	0.26	0.61	0.20	0.19
Mean	0.71	0.29	0.62	0.38	0.46	0.24	0.30
SE	0.05	0.05	0.03	0.03	0.04	0.03	0.04

TABLE 5. Within-population genetic diversity estimates for populations of wild barley.

Region/population	Mean number of alleles per locus (n_a)	Effective number of alleles (n_e)	Proportion polymorphic loci (P)	Gene diversity (H_e)
Desert				
Dimona	1.69	1.20	0.46	0.121
Ein Yarkeam	1.38	1.14	0.38	0.095
Sede Boqer	1.15	1.04	0.08	0.026
Yeruham	1.38	1.17	0.31	0.107
Jeriho	1.31	1.11	0.23	0.108
Dead Sea	1.31	1.07	0.31	0.053
Mean	1.37	1.12	0.30	0.085
95% CI (bootstrapping over populations)	(1.25–1.51)	(1.08–1.16)	(0.22–0.37)	(0.062–0.108)
Pooled desert	2.08	1.14	0.69	0.109
95% CI (bootstrapping over loci)	(1.55–2.55)	(1.02–1.28)		(0.019–0.165)
Mediterranean				
Yavne	1.23	1.03	0.23	0.028
Bet Guvrin	1.85	1.28	0.62	0.167
Jerusalem	1.31	1.17	0.23	0.108
Shoresh	1.62	1.16	0.31	0.097
Bet Shemesh	1.38	1.10	0.38	0.078
Kyriat Gat	1.62	1.19	0.38	0.122
Mean	1.50	1.16	0.36	0.100
95% CI (bootstrapping over populations)	(1.32–1.65)	(1.09–1.21)	(0.25–0.45)	(0.065–0.129)
Pooled Mediterranean	2.53	1.19	0.85	0.129
95% CI (bootstrapping over loci)	(1.90–3.20)	(1.04–1.32)		(0.050–0.210)
Overall pooled	2.85	1.17	1.00	0.115
95% CI (bootstrapping over loci)	(2.30–3.40)	(1.05–1.33)		(0.030–0.190)

TABLE 6. Genetic population structure in desert and Mediterranean regions of Israel. The total variability (H_T) is partitioned into either two levels (H_S is within population component and G_{ST} is proportion of interpopulation differentiation) or three levels (H_C is within population component, G_{CS} is proportion of interpopulation differentiation within a region, and G_{ST} is proportion of interregion differentiation).

Locus	Desert			Mediterranean			Both regions			
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}	H_T	H_C	G_{ST}	G_{CS}
<i>Est-1</i>	0.049	0.042	0.128	0.343	0.285	0.171	0.210	0.164	0.066	0.166
<i>Est-3</i>	0.495	0.351	0.291	0.484	0.234	0.516	0.510	0.292	0.041	0.402
<i>Est-4</i>	0.291	0.258	0.114	0.228	0.207	0.097	0.261	0.232	0.005	0.106
<i>Cat</i>	0.081	0.077	0.040	0.049	0.046	0.060	0.065	0.062	0.004	0.047
<i>GP</i>	0	0	—	0.049	0.047	0.031	0.025	0.024	0.010	0.031
<i>Gdh</i>	0	0	—	0.080	0.062	0.217	0.041	0.031	0.021	0.217
<i>Pgm</i>	0.080	0.062	0.217	0	0	—	0.041	0.031	0.021	0.217
<i>Pgi-1</i>	0	0	—	0.017	0.016	0.042	0.008	0.008	0.004	0.042
<i>Pgi-2</i>	0.016	0.016	0.042	0	0	—	0.008	0.008	0.004	0.042
<i>6-Pgd-1</i>	0.111	0.104	0.066	0.033	0.030	0.085	0.073	0.067	0.009	0.070
<i>6-Pgd-2</i>	0.049	0.042	0.128	0.017	0.016	0.042	0.033	0.029	0.004	0.106
<i>Mdh-1</i>	0	0	—	0.049	0.047	0.026	0.025	0.024	0.013	0.026
<i>Mdh-2</i>	0.049	0.042	0.128	0.324	0.296	0.087	0.197	0.169	0.056	0.092
Mean	0.094	0.076	0.128	0.129	0.099	0.125	0.115	0.088	0.020	0.120
95% CI (bootstrapping over loci)	(0.036–0.164)	(0.028–0.135)	(0.070–0.170)	(0.035–0.210)	(0.035–0.149)	(0.050–0.205)	(0.057–0.184)	(0.049–0.133)	(0.009–0.030)	(0.065–0.168)

after transplanting suggests that differences found in ecotype spikelet size and yield are not random (Volis et al. 2002). Therefore, at least phenotypic and reproductive traits appear to be involved in local adaptation.

The pattern of variation in phenological and morphological traits between the two regions is consistent with the hypothesis of region-specific selection. The seven traits with significant differences between regions are either phenotypic (days to awn appearance and anthesis) or reproductive (spike and awn length, number of nodes, spikelet and seed weight). Reproductive development takes longer in the Mediterranean plants, and they produce larger spikes that contain larger spikelets than the desert plants.

The amount of phenotypic variability per se in Mediterranean and desert regions might also result from region-specific

natural selection, but this is only a hypothesis. The proportion of intrapopulation variation was higher, on average, in the desert region, and plants of the two different origins (Mediterranean and desert) exhibited opposite patterns of variation for trait complexes. Mediterranean plants were twice as variable as desert plants for parameters of reproductive growth (stem and spike length) and grain filling (spikelet weight) but only half as variable for onset of reproduction. A possible explanation may employ a combined effect of directional and diversifying selection types as a result of temporal heterogeneity. (Although a higher range of micro-niches in Mediterranean populations cannot be ruled out.) Plants in a desert are subjected to unpredictable water availability and this high temporal heterogeneity may induce a spreading-risk strategy with alternative phenotypes present

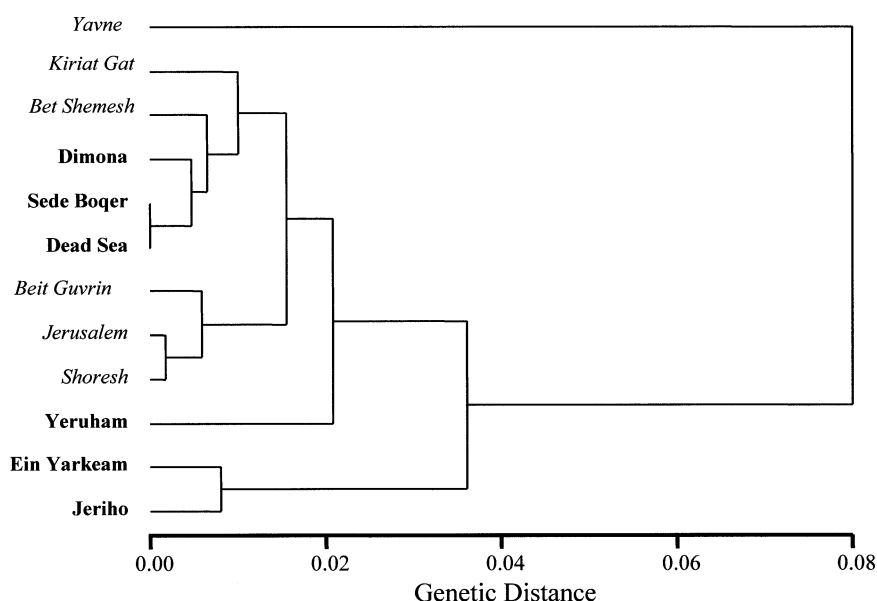


FIG. 3. Cluster analysis of 12 populations of wild barley from the desert (bold) and Mediterranean (italics) regions.

TABLE 7. The results of Ewens-Watterson test for neutrality performed on overall allele frequencies in 12 sampled populations. Sample homozygosity (F) is calculated using 1000 simulated samples. Sample size (n) was 240 for all loci.

Locus	Number of alleles (k)	Homozygosity F	SE	Approx. corresponding percentage point ¹
<i>Est-1</i>	3	0.790	0.035	60
<i>Est-3</i>	5	0.490	0.031	50
<i>Est-4</i>	5	0.739	0.031	85
<i>Cat</i>	3	0.935	0.037	60
<i>GP</i>	3	0.975	0.035	75
<i>Gdh</i>	2	0.959	0.027	65
<i>Pgm</i>	2	0.959	0.028	65
<i>Pgi-1</i>	2	0.992	0.029	90
<i>Pgi-2</i>	2	0.992	0.029	90
<i>6-Pgd-1</i>	3	0.927	0.036	85
<i>6-Pgd-2</i>	2	0.967	0.027	75
<i>Mdh-1</i>	2	0.975	0.028	80
<i>Mdh-2</i>	3	0.803	0.035	60

¹ Approximate probability of obtaining observed (or lower) homozygosity (Manly 1985).

in a population (Kaplan and Cooper 1984; Ellner 1985, 1987). The traits associated with this strategy are those enhancing spreading over the time of seed germination and plant maturation: onset of germination, seed dormancy, and start of reproduction. We found (S. Volis, unpubl. data) a significantly higher percentage of dormant seeds tested under both indigenous and alien environmental conditions in a desert as compared to a Mediterranean population. Plants from desert environments started reproduction significantly earlier than Mediterranean plants, but a transition to reproductive stage (i.e., number of days to awn appearance and anthesis) was twice as variable in the desert as compared to Mediterranean populations. However, Mediterranean populations were more variable than the desert populations in growth parameters, apparently representing the case when variability in traits not related directly to fitness has low cost and is maintained under favorable conditions.

The extent and structure of allozyme and phenotypic variation in sampled populations did not match. The Mediterranean and desert populations did not differ in amount of allozyme variation as estimated by mean number of alleles per locus, effective number of alleles, polymorphism, and gene diversity (n_a , n_e , P , and H_e). There was no segregation of the Mediterranean and desert populations on the basis of population genetic distances (Fig. 3). The proportion of interregional allozyme diversity was only 2%. The pattern of allozyme variation within each region is in accord with interpopulation mosaic structure found by Brown et al. (1978) for Israel; Nevo et al. (1986a,b) for Turkey and Iran; and Jana and Pietrzak (1988) for Jordan, Syria, Turkey, and Greece. This mosaic pattern is characterized by genetic differentiation over short geographic distances with the frequency of common alleles (> 10%) often being localized and high. However, we found no support for a hypothesis of an adaptive nature of wild barley interpopulation differences in allozyme allele frequencies as suggested by Nevo et al. (1979). The pattern of interpopulation allele distribution

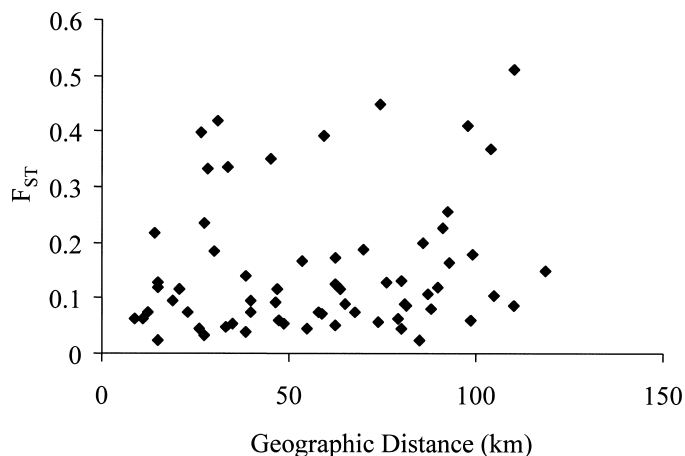


FIG. 4. Scatterplot of pairwise population F_{ST} -estimates against geographic distances separating populations.

found in this study was generated by random simulations (Ewens-Watterson test) with probability higher than 50% for all alleles; that is, the distribution of all alleles could be explained by chance alone. Rousset (1999) proposed a model of population structure involving two classes of subpopulations found in sympatry. This model predicts that under partial isolation a between-class differentiation will be detected by hierarchical analysis of population structure (F -statistics) only if habitat-related divergent selection is operating, otherwise not exceeding within-class differentiation. As soon as between-region differentiation (G_{ST}), on average, was much lower than within-region differentiation (G_{CS} ; 2% vs. 12% respectively) and in neither loci between-region variation exceeded variation among populations, region-specific selection effect on allozymes can be ruled out.

Altogether these results suggest that there is no concordance between allozyme and quantitative trait variation in wild barley; adaptation of plants originating from desert and Mediterranean environments is reflected (at least partly) in life history, phenology, and morphology; and allozymes did not provide any evidence of regional adaptation. This does not mean that all alleles are selectively neutral in wild barley. It can be that differences in phenotypic traits of desert and Mediterranean barley plants are not due to enzymatic differences, but still are the result of adaptive processes at the molecular level. In our opinion, it is misleading to interpret significant correlation of allozyme frequencies with environmental factors, as reported for many polymorphic enzymes in different species and particularly in *H. spontaneum* (Nevo et al. 1979, 1986c; Chalmers et al. 1992), as evidence of adaptation. Although in some cases this approach may be legitimate, only studies that directly examine the relationship of allele frequencies with environmental factors (either by tracing changes in molecular genotype frequencies following artificial or natural selection or by measuring fitness of different genotypes in a set of environments) can witness molecular selection.

We interpret the observed pattern of allozyme variation as due to the combination of gene flow and genetic drift and not because of local selection. However, there was no relationship between genetic distance estimated from allozyme

frequencies and geographic distance among populations, which is expected in models of population structure based on limited dispersal abilities of organisms (isolation by distance and stepping stone models; Wright 1943; Kimura and Weiss 1964). These models of regional population structure share the main assumption of the island model (Wright 1931) of a regional equilibrium, a condition of balance between loss of alleles due to drift and immigration through gene flow between populations of a region. Highly restricted dispersal distance in wild barley (usually limited for a seed to the radius of the mother plant) coupled with predominant self-pollination (>99%; Nevo et al. 1979) implies that this species' population structure should correspond to the two-dimensional stepping stone theoretical model. In this model dispersal ability is constrained by distance such that gene flow is likely to occur between neighboring populations (Malécot 1955; Kimura and Weiss 1964) leading to higher genetic similarity of geographically closer populations. The model predicts a positive and monotonic increase in genetic distance between populations with the geographic distance separating the populations. Absence of such a relationship indicates no regional equilibrium; that is, either homogenizing effect of gene flow or population differentiation through genetic drift is prevailing. Four hypothetical relationships of genetic (F_{ST}) and geographic distances were proposed by Hutchison and Templeton (1999) of which case III corresponds to what was found in this study. This case describes a lack of regional equilibrium (no significant association between pairwise F_{ST} and geographic distance) with drift more influential than gene flow (high degree of scatter between the plotted points, which is also independent of geographic distance). We found this pattern at two hierarchical levels, when considering: (1) pooled regions; and (2) each region separately. It is plausible that with a decrease in hierarchical level (from tens of kilometers to kilometers and meters) a regional equilibrium will be found due to increase in likelihood of dispersal at shorter geographic distances. Two factors may be responsible for a lack of regional equilibrium: insufficient historical time since colonization (Slatkin 1993) and transient population demography (Wade and McCauley 1988; Whitlock 1992; McCauley et al. 1995). Ecology and life history of wild barley, which is an annual ruderal species that occupies habitats with high degree of disturbance (Harlan and Zohary 1966; Von Bothmer et al. 1995) and experiences great demographic fluctuations (S. Volis, unpubl. data) leads us to hypothesize that this species may never achieve an equilibrium at a regional scale.

ACKNOWLEDGMENTS

We would like to thank A. Nurberdiev for fieldwork and R. Soto for assistance in the laboratory. We are also grateful to S. J. Tonsor and two anonymous reviewers, whose comments helped to improve this paper. This study was supported by a grant from US AID-CDR for scientific collaboration of the states of the former Soviet Union with Israel (CA-13-057).

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