

Regional Subdivision in Wild Barley Allozyme Variation: Adaptive or Neutral?

S. VOLIS, I. SHULGINA, D. WARD, AND S. MENDLINGER

From the Institutes for Applied Research, Ben-Gurion University of the Negev, POB 653, Beer Sheva 84105, Israel (Volis, Shulgina, and Mendlinger), and Mitrani Department for Desert Ecology, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel (Volis and Ward). D. Ward is currently at the Department of Nature Conservation, University of Stellenbosch, P. Bag X01, Matieland 7602, South Africa.

Address correspondence to S. Volis, Institutes for Applied Research, Ben-Gurion University of the Negev, POB 653, Beer Sheva 84105, Israel, or e-mail: volis@bgumail.bgu.ac.il.

Abstract

We examined the adaptive importance of allozyme variation in wild barley (*Hordeum spontaneum*). The test involved a nested sampling design with four population groups, each representing a different environment, and a comparison of observed allozyme variation with that expected under the assumption that allozymes are not neutral. Measurements of plant fitness in indigenous and alien environments in reciprocal introductions of seeds and seedlings in the four environments provided a guideline for the expected pattern of allozyme variation. The results showed considerable variation in both the degree of regional and population subdivision and the pattern of the subdivision among loci. The observed pattern of variation was ambiguous. Although two alleles exhibited a pattern of distribution that cannot be explained by genetic drift as a function of geographic distance, we failed to detect either a significant relationship between genetic distance and environmental similarity or any favored epistatic allele combinations across the four environments. Our results suggest that interpretation of allozyme variation in wild barley as adaptive and directly related to local environment still needs justification. Although we could not reject the null hypothesis, a proposed methodology seeking a concordance between observed and “adaptive” (i.e., expected under hypothesis that allozymes are not neutral) allozyme variation may prove to be effective in resolving the neutralist-selectionist debate when applied to other species.

Isozymes as detectable protein markers which are under the direct influence of specific gene loci have been intensively used during the last three decades for examining the role of the environment in maintaining genetic variation (reviewed in Eanes 1999; Hedrick 1986; Hedrick et al. 1976; Lewontin 1991). However, despite large efforts invested in allozyme population genetics, no study except a classic work by Allard and others on *Avena barbata* (Allard et al. 1972; Clegg and Allard 1972; Hamrick and Allard 1972, 1975) could unequivocally relate the observed allozymic variation to environmentally specific adaptive process. It has been common for studies examining the association of genetic variation with discrete environmental heterogeneity (soil type, sun versus shade, vegetative community type, etc.) to assume a priori that natural selection was occurring. Moreover, the presumed selection was often regarded as evidence against the neutral theory (Ewens 1979; Nevo 1983; Nevo and Beiles 1988), despite the fact that when

populations are sampled over space there is always a clinal pattern in one or more loci that correlates with some environmental parameter (e.g., rainfall, temperature, or salinity) (Hedrick et al. 1976). This methodology is explicit in tests for statistical association of allelic frequencies with environmental variables (Clarke 1975; Nevo 1983, Nevo and Beiles 1988) or implicit in comparative assessment of geographic variation in allele frequencies for “selective” protein and “neutral” DNA polymorphisms (McDonald 1994). The major methodological flaw of this approach is that although such association may indicate a selection effect, no conclusion of natural selection can be made without appropriate tests of individual fitness under different environmental regimes.

There are logistical limitations to the detection of spatially variable selection through direct tests of local adaptation; consequently the number of tested habitats/locations is usually small. To date, large-scale studies of

genetic structure in natural plant populations have either failed to show allozymic differentiation among ecotypes (Aitken and Libby 1994; Freiley 1993; Volis et al. 2002a; Westerbergh and Saura 1992) or, when such differentiation was reported (Heywood and Levin 1985; Lack and Kay 1988; Lönn 1993; Lönn et al. 1996; Van Rossum et al. 1997), no causal relationship between the observed pattern of variation and acting selective force(s) was demonstrated. As a result, stochastic and deterministic causes of population differentiation remained undistinguished. Nevertheless, sources of variation in spatial population structure, stochastic (gene flow and random drift) and deterministic (natural selection), are important (Heywood 1991; Linhart and Grant 1996). Rapid development of the population substructure without spatially heterogeneous selection was shown in simulation studies of isolation-by-distance models (Sokal and Wartenburg 1983; Turner et al. 1982). Moreover, local genetic differentiation through random drift and limited gene flow may produce patterns equivalent to those expected under localized selection processes (Kimura and Maruyama 1971). The interaction of selection with local gene dispersal can either reinforce or retard population differentiation, depending on whether polymorphism is independent of locality or not. When fitnesses of alleles of polymorphic loci are locality dependent, limited gene flow may enhance population differentiation (Dickinson and Antonovics 1973; Maynard Smith 1966), while selection that is locality independent will retard it (Epperson 1990).

We tested the presence/absence of spatially variable selection effects on allozyme markers. We sampled 20 populations of wild barley (*Hordeum spontaneum*) in four distinct environments in Israel, five populations per environment (group), and estimated the genetic relationships among populations. Our null hypothesis was that strong environmentally specific natural selection (= region specific) is operating directly (or indirectly through hitchhiking) on allozymes. In our study environmentally specific selection was explicit and detected de facto by reciprocal introduction of seeds and seedlings with superior fitness of transplants in indigenous as compared with alien environments (Volis et al. 2002b). Allozyme population genetic structure had to be compared with that expected under environmentally specific selection under the assumption of adaptive nature of molecular markers. If a significant discrepancy between observed and expected genetic variation patterns is found then the null hypothesis can be rejected.

As populations within each group (region) were sampled in identical or almost identical environments, and population groups represented environments chosen a priori, strong region-specific selection should lead to high genetic similarity between populations within a group. The null hypothesis can be rejected if there is no difference in the degree of similarity between populations from the same and different environments/groups. However, higher similarity of populations from the same groups could be due to natural selection, local gene dispersal, or both. To differentiate between these two requires identification of genotypes and comparison of groups. Under region-specific natural selection, groups that

are spatially distant but environmentally similar are expected to be genetically more similar than groups that are both spatially and environmentally distant. Besides, the probability of having the same genotypes favored by identical selection forces should be higher in environmentally similar groups.

Traits that are tightly related to fitness reflect the effects of predominant forces, either stochastic (genetic drift, founder events) or deterministic (natural selection). For example, the former will be the case when one or several genetically identical individuals found a population (i.e., there is no genetic variation for selection to act on). Fitness and fitness-related traits can be used for detection of the local selection by genotype-environment interaction experiments. However, allozyme and DNA genetic markers can either be uniformly affected by stochastic factors if they are selectively neutral or they may show different patterns of variation depending on the intensity of natural selection on a particular locus. One possible situation is when the overall allozyme variation pattern disagrees with what is expected under strong environmentally specific selection, but there is such a selection effect on one or a few particular loci (McDonald 1994). Therefore genetic similarity between populations and population groups must be estimated not only across all loci but for each locus as well (Eanes 1999; McDonald 1994).

Materials and Methods

Populations

Wild barley (*Hordeum spontaneum* Koch) is a predominantly autogamous annual grass (Harlan and Zohary 1966). In Israel, despite its mainly Mediterranean and Irano-Turanian distribution in steppe-like formations, wild barley penetrates into desert (<200 mm annual rainfall) and mountain (up to 1,600 m elevation) environments, where it maintains stable populations. To test our hypothesis we chose four environments/vegetation communities: desert, semisteppe batha, Mediterranean grassland, and mountain, and made seed collections in five populations in each environment. The four environments represent different conditions with respect to the amount of available water in the soil, predictability of the environment, and environmental stresses (drought and frost) (Table 1). There is a gradient of annual rainfall amount increasing from desert to mountain and inversely related to its unpredictability. In addition, in desert and mountain localities, plants are exposed to contrasting stresses (drought in the desert and winter frosts in the mountain region). A detailed description of the environments can be found in Volis et al. (2002b) and the climatic and geographic parameters used to access the environmental similarities are presented in Table 1. Altogether these four environments represent a unique spectrum of environmental effects and natural selection pressures. The last were analyzed by multilevel selection analysis of phenotypic variation and found to result in distinct sets of coadapted traits, or tactics *sensu* Stearns (1976) (Volis et al. 2002c).

Seed collection was done in 1996 in 20 localities, employing a cluster sampling design with five populations

Table I. Ecogeographical data for the four pivot populations used in an analysis of environmental similarities

Environment	Altitude (m)	Climatic parameters					
		Tm	Ta	Tj	Hu	Rd	Rn
Mountain	1,500	11	20	1	52	70	1,600
Grassland	300	19	26	10	48	50	580
Semisteppe batha	270	19	26	11	47	37	408
Desert	470	19	26	9	36	15	90

Tm, annual temperature; Ta, temperature in August; Tj, temperature in January; Hu, humidity at 1400; Rd, number of rainy days; Rn, annual rainfall (mm).

per environment (group) collected in habitats as identical as possible. Relief, slope exposition, vegetation, and soil type were kept constant in sampling localities within a group. Populations comprising a group were ≤ 5 km (mountain) or ≤ 20 km (the other three groups) from each other. All 20 populations were used in a study of population genetic structure, and 1 population from each group (pivot) was used in a test of local adaptation and a comparative study of plant life histories (Volis et al. 2002b) and phenotypic plasticity in response to water and nutrient stress (Volis et al. 2002d). Reciprocal introductions of seeds and seedlings revealed

locally adaptive genetic differentiation in life history and reproductive traits as a result of the selection process (Volis et al. 2002b); therefore the observed differences between pivot populations (at least at the phenotypic level) cannot be attributed to stochastic processes like drift or founder effect.

Electrophoresis

Nine enzyme systems encoding 17 loci were examined by starch-gel electrophoresis following the protocol and recipes of Brody and Mendlinger (1980) and Mendlinger and

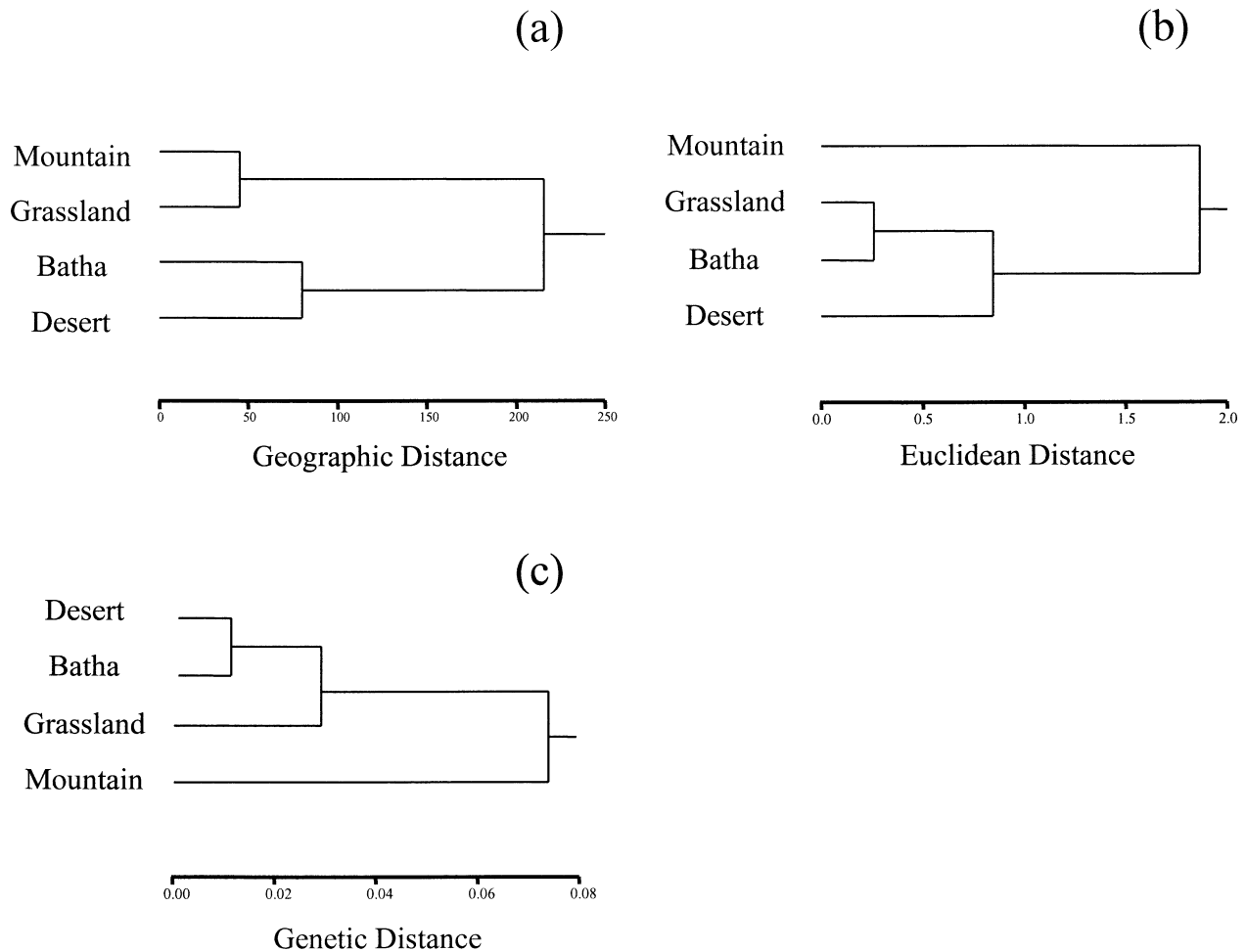


Figure 1. UPGMA dendrogram of (a) geographic distances and (b) similarities based on climatic parameters between four environments; and (c) genetic distances between four gene pools (population groups).

Table 2. The allele frequencies in four population groups of *H. spontaneum* and results of the Ewens–Watterson test performed on the population groups' gene pools for each locus

Locus/allele	Population groups				Homozygosity <i>F</i>	Corresponding percentage point ^a
	Desert	Batha	Grassland	Mountain		
<i>Acpb-1a</i>	.056	.040	—	.024	0.581	60
<i>b</i>	.136	.244	.048	.228		
<i>c</i>	.756	.652	.888	.708		
<i>d</i>	.044	.064	.048	.040		
<i>e</i>	.008	—	—	—		
Null	—	—	.016	—		
<i>Acpb-2a</i>	.016	.008	.080	.120	0.402	10
<i>b</i>	.380	.248	.528	.572		
<i>c</i>	.548	.712	.368	.212		
<i>d</i>	.032	.032	.008	.016		
<i>e</i>	.024	—	—	—		
Null	—	—	.016	.080		
<i>Cat-a</i>	.008	—	—	.008	0.976	95
<i>b</i>	.976	1.00	.976	.992		
<i>c</i>	—	—	.024	—		
Null	.016	—	—	—		
<i>Est-1a</i>	.008	.040	—	.040	0.941	75
<i>b</i>	.968	.952	1.00	.960		
<i>c</i>	.024	.008	—	—		
<i>Est-2a</i>	.032	.008	.016	—	0.609	60
<i>b</i>	.068	.088	.280	.008		
<i>c</i>	.772	.768	.576	.928		
<i>d</i>	.088	.072	.048	.040		
<i>e</i>	.040	.008	.008	.016		
Null	—	.056	.072	.008		
<i>Est-3a</i>	.008	—	—	—	0.472	35
<i>b</i>	.048	.036	.092	.864		
<i>c</i>	.888	.900	.652	.088		
<i>d</i>	.056	.064	.208	.008		
<i>e</i>	—	—	.032	—		
Null	—	—	.016	.040		
<i>Est-4a</i>	—	.008	—	—	0.774	75
<i>b</i>	.040	.016	.068	—		
<i>c</i>	.880	.904	.748	1.00		
<i>d</i>	.056	.072	.008	—		
<i>e</i>	.016	—	.176	—		
Null	.008	—	—	—		
<i>Gdb-1a</i>	—	—	.008	—	0.749	75
<i>b</i>	.112	.080	.072	.048		
<i>c</i>	.808	.840	.896	.928		
<i>d</i>	.080	.072	.024	.024		
<i>e</i>	—	.008	—	—		
<i>Gdb-2a</i>	.052	—	.024	.008	0.930	70
<i>b</i>	.924	.964	.968	.992		
<i>c</i>	.024	.036	.008	—		
<i>d</i>	—	—	—	—		
<i>GP-a</i>	.008	—	—	—	0.949	90
<i>b</i>	.976	.968	.992	.976		
<i>c</i>	.016	.032	.008	.008		
Null	—	—	—	.016		
<i>Mdb-1a</i>	.032	.032	.024	.024	0.885	80
<i>b</i>	.944	.920	.960	.944		
<i>c</i>	.008	.048	.016	.032		
<i>Mdb-2a</i>	.008	—	—	.032	0.894	65
<i>b</i>	.936	.960	.960	.948		
<i>c</i>	.056	.040	.040	.020		
<i>6-Pgd-1a</i>	.064	.080	.024	.152	0.735	70
<i>b</i>	.844	.880	.832	.792		
<i>c</i>	.084	.040	.144	.048		
Null	.008	—	—	.008		

Table 2. Continued

Locus/allele	Population groups				Homozygosity <i>F</i>	Corresponding percentage point ^a
	Desert	Batha	Grassland	Mountain		
<i>6-Pgd-2a</i>	.016	.008	.016	.152	0.961	95
<i>b</i>	.968	.976	.984	.792		
<i>c</i>	.008	.016	—	.048		
Null	.008	—	—	.008		
<i>Pgi-1a</i>	.032	.040	.008	.016	0.831	70
<i>b</i>	.896	.884	.936	.904		
<i>c</i>	.056	.076	.056	.080		
<i>d</i>	—	—	—	—		
Null	.016	—	—	—	0.847	90
<i>Pgi-2a</i>	.016	.008	—	—		
<i>b</i>	.024	.016	.024	—		
<i>c</i>	.896	.936	.888	.952		
<i>d</i>	.016	.032	.088	.048	0.708	70
<i>e</i>	.032	.008	—	—		
Null	.016	—	—	—		
<i>Pgm-a</i>	—	.012	.024	.424		
<i>b</i>	.872	.944	.960	.548	0.708	70
<i>c</i>	.104	.036	.016	.012		
<i>d</i>	.016	.008	—	—		
Null	.008	—	—	.016		

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

Zohary (1995). Sample size was 25 plants per population. Each gel contained five randomly chosen plants from each of three populations originating in different environments plus a standard. Three buffer systems—Tris-citrate (TC), Tris-malate (TM), and a Poulik discontinuous buffer (Poulik)—were used. The loci studied were acid phosphatase (*Acpb*), EC 3.1.3.2, two loci; catalase (*Cat*), EC 1.11.1.6, one locus; general protein (*Gp*), EC 4.1.1.39, one locus; glutamate dehydrogenase (*Gdh*), EC 1.4.1.2, two loci; esterase (*Est*), EC 3.1.1.2, four loci; malate dehydrogenase (*Mdh*), EC 1.1.1.37, two loci; phosphoglucomutase (*Pgm*), EC 2.7.5.1, one locus; phosphoglucose isomerase (*Pgi*), EC 5.3.1.9, two loci; and 6-phosphogluconate dehydrogenase (*6-Pgd*), EC 1.1.1.44, two loci.

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.

Data Analysis

The analyses of genetic data were performed using POPGENE version 1.31 (Yeh et al. 1998) and NTSYSpc version 2.0 (Rohlf 1998). Nei's genetic distance (Nei 1972) was calculated and used in a cluster analysis (unweighted pair group method with arithmetic mean [UPGMA]). The partitioning of genetic diversity into its three components—within populations, between populations of a group, and among groups—was accomplished using *F* statistics (Weir 1996). The following measures were calculated: total inbreeding coefficient, *F* (F_{IT}), as the correlation between alleles within individuals in the total population; *f* (F_{IS}), as the correlation between alleles within individuals within subpopulations; and θ (F_{ST}) as the correlation between alleles of

different individuals in subpopulations. In our study a three-level hierarchical analysis determined genetic differentiation among population groups (θ_{groups}), genetic differentiation among populations within groups (θ_{pop}), as well as the inbreeding coefficient of the total sample (F_{IT}) and *f* (inbreeding coefficient within populations). *F* statistics were also calculated for each population group separately. In addition, we estimated F_{IP} (population group inbreeding coefficient). We also performed Ohta's (1982) two-locus population subdivision analysis (*D* statistics). In this analysis, the variance in linkage disequilibrium is partitioned into the following components: D_{IS}^2 (variance of within-subpopulation disequilibrium), D_{ST}^2 (variance of the correlation of genes of the two loci of different gametes of one subpopulation relative to that of the total population), D'_{IS}^2 (variance of the correlation of genes of the two loci of one gamete in a subpopulation relative to that of the total population), D'_{ST}^2 (variance of the disequilibrium of the total population), and D_{IT}^2 (total variance of disequilibrium). The Ewens–Watterson test for neutrality (1,000 permutations per test) (Manly 1985) was performed for each locus to detect possible effects of selection on interpopulation allele distribution.

To compare the similarities of populations from the same groups with similarities of populations from different groups, Nei's (1972) genetic identities were calculated: 1) for all pairs of populations within a group, and 2) for five populations of a group with all other populations except these five. The mean genetic identities based on 10 pairwise comparisons (I_{within}) and 75 pairwise comparisons (I_{among}) were compared for each population group by bootstrapping with random reassignment to regenerate the original unequal sample sizes. This was repeated 1,000

times using the program RESAMPLING STATS (Simon 1995). Bootstrapping was also applied for estimating 95% confidence intervals for estimates of F statistics over loci. Genetic, environmental, and geographic distances were subjected to a Mantel test. Evaluation of the significance of each matrix correlation employed a comparison of the observed Mantel test statistic, Z , with its random distribution generated by 1,000 random permutation (using MXCOMP of NTSYSpc).

Results

Genetic Similarity

The similarity between the four environments was assessed by cluster analysis performed on six climatic parameters and one geographic parameter (altitude) (Table 1). The most similar environments were batha and grassland, desert was distinct from both, and mountain was distant from all three. However, geographically (i.e., by physical distance) batha is closer to the desert and grassland is closer to the mountain environment (Figure 1a,b).

The similarity between the four gene pools (pooled allele frequencies from 17 loci over five populations comprising a group) is shown in Figure 1c and the group allele frequencies in Table 2. Genetic similarity decreased in the following order: desert and batha, grassland, mountain. There was no significant correlation of group genetic distances with either the group geographic distances or with the group environmental similarities. However, the relationship of genetic and geographic distances approached the level of 5% significance ($r = 0.45$, $P = .085$; Mantel test), while genetic distance and environmental similarity were completely unrelated ($r = -0.10$, $P = .430$; Mantel test). Pairwise Nei's genetic identities calculated for populations comprising a group were significantly higher than those calculated for populations from different groups (except the mountain group, for which the difference was marginally significant) (Table 3). As the overall similarity between population groups may obscure the effects of genetic drift and/or selection on particular loci, we performed the Ewens–Watterson test for neutrality for each locus (Table 2). Only allozyme frequencies in four population groups at the *Acpb-2* locus had a relatively low probability of occurring by chance ($P = .10$). The allozyme frequencies in groups at the other 17 loci could be explained by chance alone (P ranged from .35 to .95).

Comparison of populations over all 17 loci (Figure 2) found that all 5 populations from the mountain region comprise a single cluster separated from the other 15 populations, while the other populations exhibited a trend of clustering in correspondence to their environments. Populations from desert and batha environments were very similar, while grassland populations were separate from them and more dissimilar from each other. The cluster analysis conducted separately for the most variable loci did not show clear regional/environmental differentiation at any locus, although a segregation of mountain populations from the

other populations was found for *Est-3* and, albeit less pronounced, for *Pgm* (Figure 3).

Population Genetic Structure

Mean θ_{group} was 0.121 and mean θ_{pop} calculated in the analysis of the entire sample was 0.104, indicating a similar degree of genetic differentiation among population groups and among populations within groups (Table 4). Only in *Est-3* and *Pgm* was the among-group component of variation substantially larger than among-population variation (0.505 versus 0.137 and 0.244 versus 0.065, respectively). θ_{pop} calculated in each population group analysis did not differ significantly from each other (Table 3).

Linkage Disequilibria

Ohta's two-locus analysis of population subdivision performed for each population group revealed consistent relationships for all four groups, viz. $D_{\text{IS}}^2 < D_{\text{ST}}^2$ and $D'_{\text{ST}} < D'_{\text{IS}}$ (Table 5). This pattern is expected under Ohta's (1982) model for population subdivision with limited migration, but not under epistatic natural selection.

Discussion

There was greater genetic similarity among populations from the same environment than among populations from different environments. In addition, the among-environment component of variation in allele frequencies was as high as among populations within environments, suggesting the importance of genetic differentiation between the four environments. Therefore our null hypothesis of strong environmentally specific natural selection cannot be rejected on the basis of the overall pattern of population genetic similarities and genetic structure. A more intricate insight into possible consequences of selection in different environments can be obtained as follows:

Genetic differentiation among four environments, if caused by natural selection, must be reflected in a distribution of allele frequencies among environments. The latter can be analyzed by the Ewens–Watterson test conducted for each locus. However, interpretation of the results of the Ewens–Watterson test has to incorporate information on environments (i.e., their degree of similarity) (Figure 1b). In only one locus (*Acpb-2*) did allozyme frequencies in the four population groups have a relatively low probability of occurring by chance ($P = .10$), but the observed differences in allele frequencies among environments (similar allele distributions for desert and batha versus grassland and mountain) (Table 2) contradict what is expected under environmentally specific selection (similar allele distributions for batha and grassland versus the other two). All other loci were found to be selectively neutral. Even in loci *Est-3* and *Pgm*, which exhibited higher among-environment variation than among populations within environments, the interregional variation could be explained by chance alone as assessed by the Ewens–Watterson test.

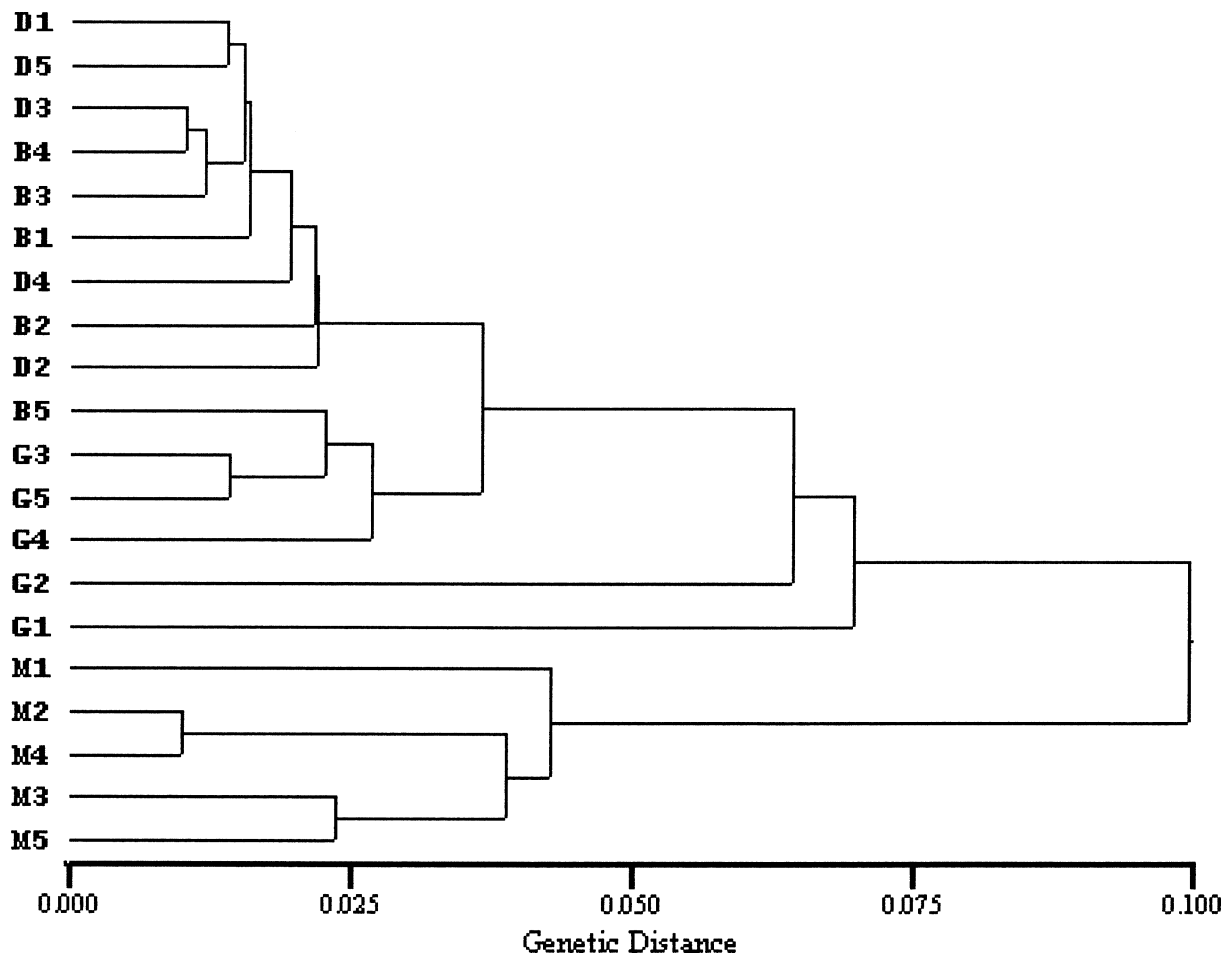
Table 3. Mean genetic identity between populations inside a group (I_{within}) and outside a group (I_{among}) and genetic population structure estimated by F statistics (f , F_{IT} , θ_{pop}) in four population groups

	Genetic identity			F statistics		
	I_{within}	I_{among}	P	f	F_{IT}	θ_{pop}
Desert	0.9813	0.9431	<.0001	0.922 (0.909–0.985)	0.926 (0.913–0.985)	0.061 (0.048–0.076)
Batha	0.9757	0.9367	<.0001	0.910 (0.873–0.972)	0.918 (0.877–0.976)	0.067 (0.048–0.086)
Grassland	0.9457	0.9054	<.0001	0.955 (0.915–0.990)	0.963 (0.923–0.932)	0.100 (0.061–0.147)
Mountain	0.9649	0.9494	.056	0.976 (0.960–0.995)	0.979 (0.961–0.995)	0.084 (0.059–0.108)

Number of pairwise comparisons was 10 for I_{within} and 75 for I_{among} . Estimates of F statistics are provided with 95% confidence interval (bootstrapping over loci).

If natural selection is environmentally induced, genetic similarity among population groups should reflect the similarity between their environments and be independent of geographic distance. Alternatively, if genetic differentiation of population groups is a result of limited gene flow and genetic drift, group similarity should be a function of the geographic distance between them. The observed pattern of interenvironment genetic similarities is ambiguous (Figures 1c and 2). High genetic similarity of

geographically close, but environmentally distant desert and batha suggests that there is or was in the recent past gene flow with either no effect or a similar effect of selection on group allele frequencies. In contrast, very low genetic similarity of geographically close and environmentally distant grassland and mountain can be either the result of genetic drift or selection. Similarly high frequencies of *Est-3b* and *Pgm-a* alleles in all five mountain populations as opposed to all other populations (Figure 3) can be equally

**Figure 2.** UPGMA dendrogram of genetic distances (Nei 1972) between 20 populations of wild barley representing four environments: mountain (M1–5), grassland (G1–5), semisteppe batha (B1–5), and desert (D1–5).

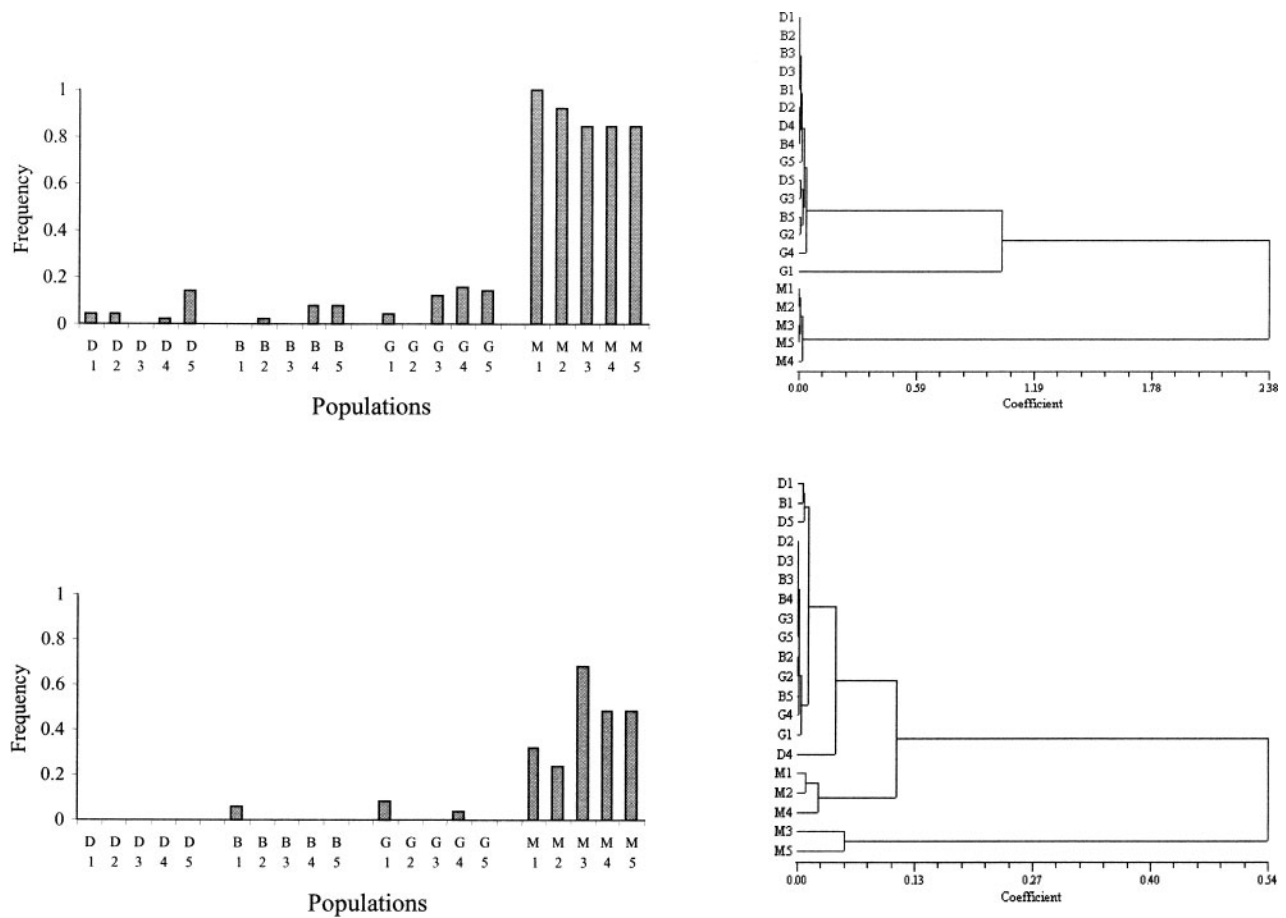


Figure 3. Population frequencies for *Est-3b* (top) and *Pgm-a* (bottom) alleles and UPGMA dendrograms of genetic distances between 20 populations of wild barley for corresponding loci, *Est-3* and *Pgm*.

Table 4. Genetic population structure for 17 loci from 20 populations of wild barley estimated by *F* statistics (*f*, *F*_{IT}, θ_{groups} , and θ_{pop}) with 95% confidence interval (bootstrapping over loci)

Locus	<i>f</i>	<i>F</i> _{IT}	θ_{groups}	θ_{pop}
<i>Acpb-1</i>	0.907	0.917	0.023	0.085
<i>Acpb-2</i>	0.885	0.909	0.089	0.130
<i>Cat</i>	0.856	0.856	0.007	-0.004
<i>Est-1</i>	1.000	1.000	0.004	0.027
<i>Est-2</i>	0.970	0.976	0.057	0.136
<i>Est-3</i>	0.892	0.954	0.505	0.137
<i>Est-4</i>	0.986	0.991	0.024	0.318
<i>GP</i>	1.000	1.000	-0.003	0.010
<i>Gdb-1</i>	1.000	1.000	-0.001	0.063
<i>Gdb-2</i>	0.834	0.838	0.012	0.011
<i>Mdb-1</i>	0.962	0.964	-0.012	0.053
<i>Mdb-2</i>	0.978	0.979	-0.010	0.042
<i>6-Pgd-1</i>	0.992	0.993	-0.007	0.109
<i>6-Pgd-2</i>	1.000	1.000	-0.006	0.010
<i>Pgi-1</i>	0.965	0.966	-0.011	0.043
<i>Pgi-2</i>	1.000	1.000	0.004	0.018
<i>Pgm</i>	0.919	0.943	0.244	0.065
Overall	0.942	0.954	0.121	0.104
95% confidence interval	(0.925-0.971)	(0.937-0.977)	(0.008-0.110)	(0.046-0.104)

Table 5. Variance components for the observed allozyme LDs (Ohta 1982)

Groups	D_{IT}^2	D_{IS}^2	D_{ST}^2	D'_{IS}^2	D'_{ST}^2
Desert	0.0219	0.0019	0.0203	0.0215	0.0004
Batha	0.0287	0.0013	0.0277	0.0283	0.0003
Grassland	0.0641	0.0019	0.0601	0.0635	0.0006
Mountain	0.0423	0.0014	0.0409	0.0418	0.0004

Number of pairs of associating alleles is 136.

well explained by fixation following genetic drift or by adaptive advantage in the mountain environment.

The last step in our test of the null hypothesis is the use of D statistics (Ohta 1982) to analyze the causes of non-random associations of alleles: epistatic natural selection and random genetic drift. Strong multilocus associations are expected in predominantly self-pollinating species (Brown et al. 1977) and geographically widespread multilocus organization with a variance 80% higher than expected under random association was demonstrated in Israeli populations of wild barley (Brown et al. 1980). Ohta's model of linkage disequilibrium in finite subdivided populations at equilibrium predicts increasing differentiation of gamete types among subpopulations (correlation of nonallelic genes within a subpopulation) under limited migration as a consequence of random genetic drift. On the other hand, if epistatic environmental (= region specific) selection is responsible for linkage disequilibrium but not for local differentiation, the gametes with favorable combinations of alleles will be selected for in every subpopulation. The ratios D_{ST}^2/D_{IS}^2 and D'_{IS}^2/D'_{ST}^2 are measures for testing which of two factors (epistasis or limited migration) is responsible for the observed linkage disequilibrium. Spatial and temporal heterogeneity of environments occupied by natural populations precluded wide applicability of Ohta's test, because of possible fine-scale local selection. In our study, where environmental types were chosen a priori and sampling of populations was done within each environment in near identical habitats, Ohta's model is appropriate. The D analysis, separately done in each population group, did not reveal any allele combination that was favored in a particular environment across all five populations. This was also true for *Est-3b* and *Pgm-a* alleles. This indicates that the observed linkage disequilibrium is due to limited gene flow and genetic drift only, without a noticeable contribution by epistatic selection in any environment.

Together the tests conducted revealed that 1) the overall pattern of allozyme variation among and within environments is not congruent with what is expected under strong environmentally specific selection; 2) this pattern corresponds in general to what is expected under isolation by distance; and 3) although no allele combinations (linkage disequilibrium) were favored in any environment, two alleles, *Est-3b* and *Pgm-a*, exhibited distributions that may indicate their selective advantage in the mountain environment. In a reciprocal introduction of barley seeds originating in four environments, we found a significantly higher survival percentage of indigenous plants at the mountain site as

compared with three alien ecotypes (Volis et al. 2002b). All seedlings of indigenous and introduced ecotypes that survived a winter set seeds and their yield did not differ (Volis et al. 2002b). Therefore, if high frequencies of *Est-3b* and *Pgm-a* alleles resulted from mountain-specific natural selection, we can expect 1) the corresponding enzymes to be involved in cold- or frost-related metabolism, and 2) the allozymes to have different fitness effects in cold-/frost-different natural or experimental conditions. The authors are unaware of any study indicating either of these and leave it as an open question.

Our results agree with previous findings that wild barley allozyme variation in Israel shows both regional and local patterns (Brown et al. 1978; Nevo et al. 1979), although we found a lower contribution of interregional and interpopulational (within regions) components of allozyme diversity (12% and 10%, respectively) than Brown (Brown et al. 1978) (17% and 32%, respectively). The lower interregional diversity found in our study as compared with Brown et al. (1978) is not surprising, as we covered a narrower species environmental and geographic range. The difference in the interpopulational component of variation between the two studies appears to result from the closer proximity of populations to each other in our study, as well as because a more subjective and a posteriori grouping of populations in Brown et al. (1978). For example, the geographic group of the Golan Heights in Brown's study included three locations with altitudes ranging from 330 m (mild winter) to 1,530 m (cold winter with frosts), clearly distinct environments. There is also a similarity in allozyme variation detected for *Est* and *Pgm* in our study and the study of Nevo et al. (1979), where the area of Mount Hermon was similarly distinct from other regions. However, our study does not support a suggestion by Nevo et al. (1979, 1986a,b) on the adaptive nature of allozyme variation in wild barley. The adaptive significance of observed allozyme variation was surmised by Nevo et al. from correlations with environmental parameters with no demonstrative causal relationship. Our study used a different approach that seeks a concordance between observed allele variation and allele variation expected under specific local selection as detected by fitness-related traits. This approach showed that most if not all allozyme variation in populations of wild barley is nonadaptive, resulting from processes other than natural selection.

Summarizing our findings, we can state that there is an overall disagreement between observed and expected allele

variation. However, high frequencies of two alleles, *Est-3b* and *Pgm-a*, in one of four regions (Mount Hermon) is difficult to explain by genetic drift only as a function of geographic distance. Although we cannot completely reject the null hypothesis, the results encourage the search for stochastic demographic and environmental causes of subdivided population structure in wild barley determining or related to seed/pollen dispersal.

The higher amount of gene flow found in the more xeric desert and batha populations as compared to the more mesic grassland and mountain populations is interesting. One possible explanation for this phenomenon could be the traditional migratory behavior of the flocks of sheep and goats in the four regions. The desert and batha populations represent the Negev desert and Shefela Hills regions, respectively, which were until relatively recently primarily populated by seminomadic or nomadic herdsman. Many of these herdsman would winter their flocks (winter is the rainy season in Israel) in the northern Negev desert where grazing and water were plentiful in the wadis and springs of the region (as many Bedouin still do today) (Degen et al. 2000). In the spring, mature spikelets are often caught in the fur of sheep and goats, which can cause gene flow from one wadi to another. In addition, in the spring, many nomads moved their flocks north to the batha region where grazing and water were available during the dry summer season (Degen et al. 2000). This would be a logical highway for gene flow between the desert and batha. A distribution of alleles among the desert and batha (15 alleles present in the desert and not in the batha versus only 4 in reverse, $\chi^2 = 6.3$, $P < .05$) provides indirect support for this possible explanation. In northern Israel, most people lived in towns and villages and grazed their flocks near their homes year-round. Thus no major mechanism was present for gene flow to occur over large distances. The effect of local grazing, absence of seed dispersal by runoff (it is one-directional from Mount Hermon downward), and small area size may be responsible for the distinct difference between the mountain gene pool and the other regions. As a result, genetic drift or founder events in the past could evolve, causing near fixation of some alleles.

Acknowledgments

This study was funded by grant no. 86293101 from the Israel Academy of Science. This is publication no. 380 of the Mitrani Department for Desert Ecology.

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Received May 29, 2002

Accepted March 23, 2003

Corresponding Editor: James L. Hamrick