

# The Influence of Space in Genetic-Environmental Relationships When Environmental Heterogeneity and Seed Dispersal Occur at Similar Scale

S. Volis,<sup>1,\*</sup> Y. Anikster,<sup>2,†</sup> L. Olsvig-Whittaker,<sup>3,‡</sup> and S. Mendlinger<sup>1,§</sup>

1. The Institutes for Applied Research, Ben-Gurion University of the Negev, POB 653, Beer Sheva 84105, Israel;

2. Department of Plant Sciences and Institute for Cereal Crop Improvement, Tel Aviv University, Tel Aviv 69978, Israel;

3. Israel Nature and National Parks Protection Authority, 3 Am Ve Olamo Street, Givat Shaul, Jerusalem 95463, Israel

Submitted November 11, 2002; Accepted June 10, 2003;  
Electronically published February 13, 2004

Online enhancement: figure.

**ABSTRACT:** We tested the importance of microenvironmental topographic parameters as predictors of emmer wheat genetic variation using three classes of single-locus (or at most several-loci) genetic markers (allozymes, glutenins, and qualitative traits) and two classes of markers of polygenic inheritance (phenological and morphological traits). Canonical correspondence analysis (CCA) and redundancy analysis (RDA) detected a significant effect of spatially structured environmental variation on genetic differences between plants for allozymes, glutenins, and quantitative morphological and phenological traits. However, after removing a spatial component of variation in partial CCA and partial RDA, the relationship of the remaining environmental variation with these genetic markers could be explained by chance alone, allowing us to rule out microniche topographic specialization in emmer wheat. Topographic autocorrelation exhibited a certain degree of similarity with genetic marker autocorrelation, indicating similar scales of environmental heterogeneity and seed flow. The detected population genetic structure agrees with one expected under isolation by distance as a result of limited gene flow. A negative relationship of genetic similarity with the logarithm of distance between plants was detected for both molecular markers and quantitative traits, which differed in the strength but not the pattern of association.

**Keywords:** genetic-environmental relationship, autocorrelation, canonical correspondence analysis, redundancy analysis, habitat selection, isolation by distance.

A major task of population genetics is the determination of the pattern of genetic differentiation in a population and its interpretation as due to either nonselective processes related to gene flow through seed/pollen dispersal (genetic drift, founder effect) or abiotic/biotic environmental effects. When an environment is heterogeneous and genetic variation within plant populations is spatially structured, it may either indicate habitat selection or be a result of the combined effects of limited movement of plant pollen and seeds, inbreeding, and genetic drift (Levin and Kerster 1974; Heywood 1991; Linhart and Grant 1996).

The conditions for maintaining genetic polymorphism in a spatially heterogeneous environment were analyzed in several models (Levene 1953; Maynard Smith 1970; Maynard Smith and Hoekstra 1980; Hoekstra et al. 1985; Garcia-Dorado 1987; Hedrick 1990, 1998) and were found to be fairly restrictive (requiring selective differences to be high or a heterozygote in all habitats to have a higher fitness than the arithmetic mean of the homozygotes) unless genotype-specific habitat selection is incorporated (Hedrick 1990). The selective variation can be maintained in a spatially heterogeneous environment without heterozygote advantage in both random mating and predominantly selfing populations (Hedrick 1998). Regardless of the mating system, a subtle genotype-specific habitat preference may be sufficient for long-term maintenance of polymorphism, especially if gene flow is limited (Hedrick 1998). However, it is not easy to recognize a true habitat-specific selection effect in observed population structure because several simulation studies have demonstrated that population substructure developed under restricted gene flow can be indistinguishable from one produced by spatially heterogeneous selection (Turner et al. 1982; Sokal and Wartenburg 1983). Many studies considered the association

\* Corresponding author; e-mail: volis@bgumail.bgu.ac.il.

† E-mail: aniksty@post.tau.ac.il.

‡ E-mail: linda.whittaker@nature-parks.org.il.

§ E-mail: mendling@bgumail.bgu.ac.il.

of genetic variation with discrete or clinal environmental variation to be a sufficient criterion for assigning population substructure as due to natural selection. Absence of such an association or failure to detect relevant environmental homogeneity was usually taken as evidence of local gene dispersal.

However, the problem of distinguishing effects of local genetic drift and selection in spatial population genetic structure is often complicated by the presence of an autocorrelation effect in both genetic and environmental variation. Although several empirical studies tested effects of environmental variables on genetic structure by either excluding neighboring effects by appropriate sampling or accounting for spatial autocorrelation by appropriate statistical analysis (Peakall and Beattie 1995; Prentice et al. 1995; Angers et al. 1999), in most recent studies the inferences about the determinants of the observed genetic structure were largely speculative. The main reason for this was the complicated relationship between environmental and spatial variation, which includes complete congruence when seed and/or pollen dispersal are fully determined by environmental heterogeneity (e.g., availability of soil pockets among rock outcrops), partial redundancy when both environmental and spatial variation exhibit autocorrelation of similar scale, and total segregation of environmental and spatial effects on genetic variation if environmental heterogeneity and seed/pollen dispersal are unrelated and of distinctly different scales.

Among statistical methods applied in testing genetic-environmental relationships, multivariate ordination techniques suit the goal of extracting pure spatial and pure environmental variation and testing their relationship to genetic variation. Canonical correspondence analysis (CCA) and redundancy analysis (RDA; ter Braak 1986) are multivariate constrained ordination techniques able to extract the major gradients in data (dependent variables) that can be attributed to the measured explanatory (independent and usually environmental) variables. They are the most powerful current multivariate constrained ordination analyses and are widely used in ecology and vegetation science. Ter Braak (1988a) first investigated genetic-environmental relationship by CCA (using data from McKecknie et al. 1975), but only a few studies since then applied CCA to genetic data (Bayer 1992; Volis 1995; Angers et al. 1999; Gram and Sork 2001). A particular strength of CCA and RDA for analysis of genetic-environmental relationship is their ability to remove the effect of undesirable variables (covariables) by regression-based covariance analysis prior to the analysis itself. This procedure is called partial canonical correspondence analysis (pCCA) or partial redundancy analysis (pRDA; ter Braak 1988b). It is possible, by virtue of CCA and pCCA (or RDA and pRDA), to measure the fraction of the variation in the genetic matrix explained

either by the environmental variables alone or the spatial structure of the data alone as well as the fraction of the variation shared by both sets of variables (Borcard et al. 1992).

Emmer wheat *Triticum turgidum* L. ssp. *diccocoides* (Körn.) Thell. is a locally abundant plant species in northern Israel that occupies a range of dissimilar ecological niches over a relatively short area, which allows for testing the relationship between genetic and environmental variability. It has been hypothesized that wild emmer prefers the microhabitat of soil pockets among tall rocks because of a favorable moisture regime and a lack of competition or refuge from grazing pressure (Aaronsohn 1909). However, emmer wheat occupies a spectrum of microhabitats, including flat or sloping areas without intensive rock outcrops where emmer competes with tall perennial forbs, annual and perennial grasses, and the shelter of spiny shrubs (Harlan and Zohary 1966). Habitat differences caused by differential soil moisture, competition, and grazing intensity can potentially lead to microscale natural selection, and several studies on wild emmer have claimed to find evidence of microhabitat specialization detectable at the molecular level (Nevo et al. 1988a, 1988b, 1991; Li et al. 1999, 2000a, 2000b).

The study by Nevo et al. (1991) of association of allozymic population genetic structure with topographically defined habitats and topographic variables themselves is of particular interest for us because it allows application of CCA and RDA analyses to separate pure environmental (topographic) versus pure spatial components of environmental variation in testing for genetic-environmental variation; the same data set used by Nevo et al. (1991) is also used in this study and is compared with the data from four other marker classes analyzed in the same way; and a methodology by which Nevo et al. (1991) came to the conclusion of microniche specialization at the molecular (allozyme) level can be verified by other potentially statistically more powerful approaches. The methodology used by Nevo et al. (1991) included identification of four major topographically and vegetatively defined habitats and 11 subhabitats, estimation of differentiation among habitats and subhabitats across loci, and Spearman rank correlation and stepwise multiple regression analyses to test for relationships between expected heterozygosity (He), polymorphism (P), and allele frequencies in subhabitats and vegetative/topographic variables.

The data used in this study, including those analyzed by Nevo et al. (1991), came from an elaborate and extensive genetic study conducted on a population of emmer wheat in northern Israel during 1984–1998 (Anikster et al. 1997). In that study, the sampling strategy used was designed to encompass maximum environmental variation (i.e., different slope exposition, altitude, rock cover, etc.).

However, since sampling was done in linear transects with approximately equidistantly located points, there could have been a strong autocorrelation component. The latter determined the goals of the present study, which included investigation of the environmental-genetic relationship in a data set where the spatial component was explicit, analysis of population genetic structure at two spatial scales, and comparative assessment of the fine-scale environmental effect on different genetic markers.

## Material and Methods

### *Study Site and Species*

*Triticum turgidum* var. *diccoides* (genome AABB,  $2n = 4x = 28$ ) wild emmer wheat is a predominantly selfing grass and the tetraploid progenitor of most cultivated wheats (Feldman 1976). It is distributed throughout the western part of the Fertile Crescent (Harlan and Zohary 1966), with a center of distribution in the catchment area of the Upper Jordan Valley in steppelike herbaceous formations of the *Quercus ithaburensis* open-park forest belt (Zohary 1973). A detailed description of the study site, which lies 1 km west of Kibbutz Ammiad (fig. 1), including topography, climatic conditions, soil, and vegetation, is given in Anikster and Noy-Meir (1991) and Noy-Meir et al. (1991). The bedrock is hard limestone, which produces rugged rock outcrops with red terra rossa soil. The climate is typical Mediterranean, with a mean annual precipitation of 589 mm (covering the years 1947–2000). The vegetation is Mediterranean grassland, with wild emmer wheat being relatively common. The altitude ranged between 240 m and 350 m above sea level.

### *Sampling Design*

A single-plant seed collection was made in 1984 at 250 sampling points arranged along four linear transects (fig. 1; Anikster and Noy-Meir 1991). Single spikes were collected along the transects at intervals of 3–5 m, with points of collection marked by pegs. Transect A starts along a north-facing slope, crosses a wadi, and continues along a south-facing slope and a ridge (points 1–90). Transect B starts atop the ridge and continues along an east-facing slope down to the wadi (points 91–150). Transect C (points 151–180) is located on a south-facing slope and differs from the other transects in its karstic deeply creviced rock formations and higher soil moisture. Transect D (points 181–230) is located in a valley and is less rocky than the other transects. Additional points were sampled later in Transects B, C, and D but are excluded from the analysis in this study because of violation of linear sequence of the transect sampling points.

A set of environmental variables was measured at marked sampling points. From this set we used only the measurements taken at all 230 sampling points: distance to base of nearest rock in four compass directions (DBN, DBE, DBS, DBW), angle and distance to top of nearest rock in four compass directions (ATN, ATE, ATS, ATW and DTN, DTE, DTS, DTW), rock type (RT), percentage of rock cover (PR), rock height (RH), width of soil pockets (PW), and accessibility to grazers due to rockiness (ACC). Of these, RT is a nominal variable, represented by five classes (small rocks up to 50-cm diameter; rough perforated rocks; rough sharp-edged rocks; smooth rock blocks; smooth flat rocks); PR, RH, PW, and ACC are ordinal variables including five classes each; and the rest are continuous variables.

In addition to topographic position and microrelief data, the sampling points were also analyzed for associated vegetation, soil depth, soil moisture, and soil nitrogen and were classified into four major habitat units: valley (including also lower slopes), ridge (various slopes and aspects), north (a north-facing slope), and karst (with deeply dissected rock relief; Noy-Meir et al. 1991). Noy-Meir et al. (1991) used a classification that employed an initial topographic subdivision by slope and aspect followed by floristic-ordination analysis. Noy-Meir et al. (1991) found a close association between floristic composition and soil moisture with rock/soil microrelief (e.g., rock cover and height) and came to the conclusion that macrotopographically defined habitats differ in vegetation because they differ in microtopographic patterns created by rock outcrops and soil pockets, with the latter affecting vegetation through soil moisture and evaporation. In the present study, we limited the number of environmental variables for analysis of genetic-environmental relationship to only microrelief variables because other variables either are interrelated with them (floristic abundance and composition, soil moisture) or were taken at selected localities (soil nitrogen, soil depth).

### *Genetic Markers and Quantitative Traits*

The seed samples collected in 1984 were propagated in a nursery to produce lines of progeny that are genetically identical with their seed parents (emmer wheat is a predominantly selfing species with an outcrossing rate of about 0.005; Golenberg and Nevo 1987). The list of markers and traits analyzed is presented in table 1. Screening of allozyme variation was done by starch gel electrophoresis (Nevo et al. 1991), analysis of high molecular weight glutenin variation was done by sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (Felsenburg et al. 1991), and qualitative and quantitative trait data were obtained from  $S_1$  accessions (seeds of self-pollinated parent plant)

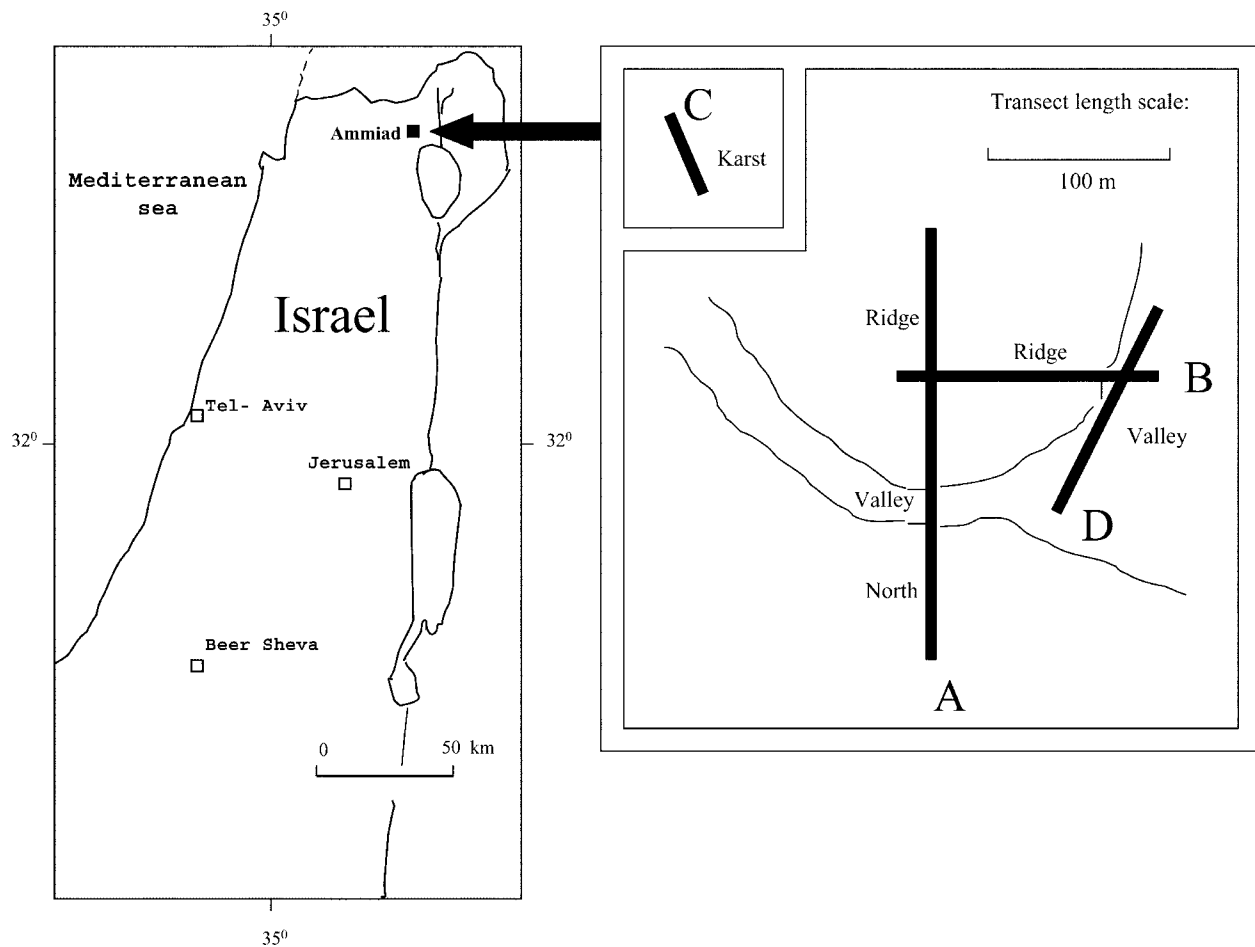


Figure 1: Map showing the study site location and a diagram of sampling transects and habitat units (from Anikster et al. 1991, with changes)

grown in a single year in a nethouse nursery at Tel Aviv University (Anikster et al. 1991). In the latter experiment, 230 plants (one individual per accession) were grown under standardized conditions. Only polymorphic allozyme loci with frequency of the most common allele  $<0.95$  (10 out of 43 screened) were used in the analyses.

#### Data Analysis

Five sets of data (allozyme and glutenin markers and qualitative, phenological, and morphological traits) and the environmental topographic data (one datum per sampling point per marker/topographic variable) were analyzed using multivariate ordination techniques (CCA and RDA) and spatial analyses. In addition, allozymes and quantitative traits were subjected to population subdivision analysis.

Population subdivision analysis was applied to allozyme

and quantitative data to estimate the amounts of variation among the habitats and transects and among five classes of nominal/ordinal topographic variables. Because sampling was done in transects having more than one habitat (except transect C), it is of interest to compare the amounts of interhabitat and intertransect variation. In both divisions, pure environmental and pure spatial components are present, but either first habitats or second transects prevail. We also examined subdivision at the microhabitat scale using topographic variables RT, RH, and ACC (the latter were identified by CCA/RDA as the main determinants of environmental-genetic relationship). The  $\theta_{ST}$  estimates were obtained following Weir and Cockerham (1984) as implemented in POPGENE version 1.31 (Yeh et al. 1998). The  $Q_{ST}$  estimates were calculated assuming complete selfing as  $Q_{ST} = \sigma_{among}^2 / (\sigma_{among}^2 + \sigma_{within}^2)$ , where  $\sigma_{among}^2$  is the among-population variance component and  $\sigma_{within}^2$  is the among-genotype (within-population) variance

**Table 1:** Genetic markers, their abbreviations, and number of alleles (allozymes and glutenins) or states (qualitative traits)

Genetic markers	Abbreviations	Number of alleles/states
Allozyme encoding loci:		
Acid phosphatase 3	Acp-3	3
Esterase 5 A	Est-5A	3
Esterase 5 B	Est-5B	5
Glucosidase A	Gluc-A	2
Malate dehydrogenase 1 A	Mdh-1A	2
Lipoamide diaphorase 2 A	Nadh-2A	2
Lipoamide diaphorase 2 B	Nadh-2B	2
Peptidase 1 B	Pept-1B	2
Phospho-glucose isomerase A	Pgi-A	3
Phospho-glucose isomerase B	Pgi-B	2
Glutenin encoding loci:		
Glu-A1-1	...	4
Glu-A1-2	...	2
Glu-B1-1	...	5
Glu-B1-2	...	7
Qualitative morphological traits:		
Color of first leaf sheath at emergence of the second leaf	SH-CO	3
Pubescence of first leaf sheath	SH-PU	3
Shape of first leaf at emergence of the second leaf	L1-SH	2
Angle between first flagleaf and shoot axis at onset of anthesis	T1-AG	2
Growth habit at anthesis	GRH	3
Spikelet color	SP-CO	2
Quantitative phenological traits:		
Days from sowing to emergence	EMR	...
Number of days from October 1 to appearance of first tiller	T	...
Days from March 1 to awn emergence on first tiller	T1-AW	...
Days from March 1 to beginning of anthesis on first tiller	T1-AN	...
Days from March 1 to ripening of the first spike	S1-HR	...
Quantitative morphological traits:		
Number of tillers at emergence of sixth leaf, including main shoot	#T	...
Length of flag leaf (mm) on first tiller at beginning of anthesis	T1-LE	...
Width of flag leaf (mm) on first tiller at beginning of anthesis	T1-WI	...
Number of spikes per plant	#S	...
Length of first spike (mm)	S1-LE	...
Number of spikelets on first spike	#SP1	...
Weight of first spike (g)	S1-WT	...

component (Bonnin et al. 1996). Since the 230 genotypes, each representing a different sampling point, were not replicated, we could not estimate the environmental variance and remove it from the total phenotypic variation and thus estimate trait heritabilities ( $h^2$ ). The latter, however, should not influence the  $Q_{ST}$  estimate under the assumption that among- and within-population components of environmental variance are proportional. Moreover, since we are most interested in a comparison of  $Q_{ST}$  values obtained using different subdivision criteria (habitats and topographic parameters vs. transects) rather than in  $Q_{ST}$  per se, our approach is justified even if this assumption is not met. To distinguish our estimate from the purely

genetic  $Q_{ST}$ , we designated it as  $Q'_{ST}$ . The 95% confidence intervals for  $\theta_{ST}$  and  $Q_{ST}$  were estimated by bootstrapping with random reassignment to regenerate the original sample sizes repeated 1,000 times. This was done using the program RESAMPLING STATS (Simon 1995).

A test for selective neutrality of allozyme loci was done as described in Vitalis et al. 2001 using program Detsel (Vitalis et al. 2002). The expected joint distribution of test estimates  $\hat{F}_1$  and  $\hat{F}_2$  was generated by 10,000 coalescent simulations for each population (i.e., habitat) pair. The "95% probability" regions of expected distribution for several sets of nuisance parameter values were conditioned on the number of allelic states in the pooled sample  $\geq 2$ .

The scatterplot of the observed values of  $\hat{F}_1$  and  $\hat{F}_2$  were superimposed over the 95% probability region to identify outlier loci. A locus can be considered experiencing the effect of selection if it is consistently found outside the 95% probability region in all pairwise comparisons (Vitalis et al. 2001, 2002). The following nuisance parameters were tested in different combinations: the mutation rate  $\mu$  0.001 and 0.0001; the ancestral population size  $N_e$  1,000, 5,000, and 10,000; the population size before the split  $N_0$  50 and 100; and time before the split  $\tau_0$  100 and 150. The time of divergence  $\tau$  was set to 50.

The effect of environmental (topographic) variables on allozyme, glutenin, qualitative, phenological, and morphological trait variation was tested by either CCA or RDA, using version 4.02 of the CANOCO program (ter Braak and Smilauer 1998). Genetic data formed five matrices of dependent variables (26 for allozymes, 18 for glutenins, 32 for qualitative traits, five and six for phenological and morphological traits). The first three classes were dummy (presence-absence) variables, and the other two classes were continuous variables. Environmental topographic data comprised a single matrix with 20 independent variables, including dummy, ordinal, and continuous. The dummy variables (allozymes, glutenins, and qualitative traits) were analyzed by CCA, and the continuous variables (quantitative traits) were analyzed by RDA. A choice between these two analyses was done according to ter Braak and Smilauer (1998), assuming either a unimodal model (CCA) or linear model (RDA) for the relationship of marker response with the ordination axes. CCA and RDA measured the amount of genetic variation (sum of canonical eigenvalues) explained by a set of environmental variables and assessed the statistical significance of environmental effect by Monte Carlo permutation test with 1,000 permutations. The environmental variables contributing most to the explanation of genetic variation were selected using a forward selection procedure of CANOCO, with a cutoff point of  $P = .10$  (default).

Since a relationship of genetic variation with environmental (topographic) heterogeneity may be apparent and may be caused by partial redundancy of environmental variability and the spatial pattern of sampling points' distribution, we applied the method of variation partitioning (Borcard et al. 1992) to estimate the following components of variation: nonspatial environmental effects (i.e., variation that can be explained by the environmental variables independent of the spatial structure); spatial structuring that is not shared by the environmental variables; spatial pattern of variation that is shared by the environmental variables; and variation explained neither by spatial structure nor by environment. Technically, the variation partitioning is done by two CCA, two partial CCA, and one correspondence analysis (CA; Borcard et al. 1992) or by

two RDA, two partial RDA, and one principal components analysis (PCA). The CA and PCA estimate the total amount of variation (sum of all eigenvalues) in a genetic matrix. The nonspatial environmental effect is computed by pCCA or pRDA of the genetic matrix, constrained by the environmental matrix; four linear transects are entered as covariables and remove the between-transect variation and possible spatial autocorrelation. In contrast, the pure spatial component of variation is estimated by pCCA or pRDA of the genetic matrix, constrained by the spatial matrix (four linear transects); the environmental variables are entered as covariables. Two separate CCA or RDA ordinations estimate the environmental and spatial effects on the genetic matrix without removal of a counterpart variation; they are used to obtain the component of variation in a genetic matrix that is shared by the spatial and environmental data. The variation that remains after subtracting the pure environmental component is the pure structural component, and the shared component is an unexplained variation.

To investigate the scale of spatial effects on genetic variation (nearest-neighbor effect) and on environment (environmental grain or patchiness), we performed an autocorrelation analysis on the same data set as above using a unified statistical procedure. This was done by calculating autocorrelations for lag 1 through lag  $k$ , in which lag 1, for example, is the Pearson correlation of adjacent sampling points, lag 2 is the correlation between sampling points one point apart, and so forth. In order to reduce the number of variables subjected to autocorrelation analysis and because Pearson correlation requires data to be continuous, we conducted PCA by CANOCO (ter Braak and Smilauer 1998) on each data set and used the first two principal components of variation in each marker class as well as in topographic variation for autocorrelation analysis. The quantitative traits and continuous environmental variables were log transformed and standardized prior to PCA as implemented in CANOCO (Borcard et al. 1992; ter Braak and Smilauer 1998). Since the autocorrelation analysis was conducted for each transect separately, and we wanted to use a unified range of autocorrelation distance classes across transects, the range of lags was limited to 10 and of approximate distance (in meters) to 40. The PCA scores for the sampling points in each transect at corresponding separating distances and the mean transect values were used in a regression analysis against the logarithm of distance between plants. Since the distribution of emmer wheat is not homogeneous and the distance separating adjacent plants in transects varied between 3 and 5 m, we used an average of 4 m as an approximated measure of distance. This allowed us to test for a negative linear relationship between pairwise genetic similarity and logarithm of distance between plants as pre-

**Table 2:** Intergroup component of variation estimated with  $F_{ST}$  and  $Q'_{ST}$  (allozymes and quantitative traits, respectively) followed by 95% CI (bootstrapping over loci)

Markers	Intergroup component of variation				
	Habitats	Transects	RT	RH	ACC
Allozymes:					
AcpH-3	.014	.017	.039	.036	.033
Est-5A	.216	.142	.100	.137	.112
Est-5B	.050	.031	.101	.040	.054
Gluc-A	.215	.175	.136	.154	.133
Mdh-1A	.157	.174	.087	.090	.165
Nadh-2A	.255	.073	.138	.081	.031
Nadh-2B	.072	.067	.060	.076	.075
Pept-1B	.030	.028	.023	.015	.054
Pgi-A	.047	.067	.076	.024	.125
Pgi-B	.437	.194	.252	.077	.137
Overall	.167	.108	.116	.077	.104
95% CI	(.082–.220)	(.063–.132)	(.067–.146)	(.047–.101)	(.065–.121)
Quantitative:					
EMR	.005	.012	.005	0	0
T	.070	.069	.052	0	0
T1-AW	.103	.072	.038	.061	.043
T1-AN	.150	.105	.057	.073	.028
S1-HR	.295	.294	.156	.085	.064
#T	0	0	0	0	0
T1-LE	.233	.177	.069	.061	.102
T1-WI	.349	.379	.170	.238	.303
#S	.013	.026	0	0	0
S1-LE	.150	.150	.017	.014	.073
#SP1	.174	.179	.145	.081	.063
S1-WT	.077	.063	.038	.028	0
Overall	.135	.127	.062	.053	.056
95% CI	(.075–.193)	(.071–.197)	(.031–.098)	(.023–.093)	(.020–.106)

Note: Groups were defined at large (habitats and sampling transects) and small (RT, RH, and ACC) spatial scale. CI = confidence interval, RT = rock type, RH = rock height, ACC = accessibility to grazers due to rockiness.

dicted under isolation by distance (IBD; Rousset 1997, 2000; Hardy and Vekemans 1999).

The allozyme data were also analyzed following Hardy et al. (2000) to obtain correlograms for each locus and a multilocus correlogram. The procedure included constructing a matrix of pairwise correlation coefficients for each pair of individuals  $i$  and  $j$ , calculated as  $[(p_i - \bar{p})(p_j - \bar{p}) / \text{Var}(p)] + 1/(n - 1)$ , where  $p$  values stand for individual frequencies of a particular allele (either 0, 0.5, or 1),  $\bar{p}$  and  $\text{Var}(p)$  are the estimated population (i.e., transect) average and variance, respectively, and  $n$  is the total number of individuals. The pairwise correlation coefficients of all possible pairs of individuals given sampling points apart were regressed against the logarithm of an approximated measure of distance as above. Following Hardy et al. (2000), we designated the average correlation coefficients as  $I(d)$  and used the negative of the slope ( $-b$ )

as an estimate of the magnitude of genetic structure under IBD. Multiallelic averages for each locus were obtained by weighting the contribution of each allele by  $p(1-p)$ , and the multilocus average was calculated by weighting the result for each locus by its polymorphic index  $\sum p(1-p)$ .

## Results

### *Population Subdivision Analysis*

Although the amount of interhabitat variation for allozymes was substantial (16.7%), it did not differ significantly from the amount of intertransect variation (10.8%) or from the amount of intergroup variation, with groups being either rock type, rock height, or accessibility to grazers (table 2). The analysis of variation across allozyme

**Table 3:** Canonical correspondence analysis (CCA), partial canonical correspondence analysis (pCCA), redundancy analysis (RDA), and partial redundancy analysis (pRDA) of variation in five classes of genetic markers

Variables	Sum of all canonical axes		Sum of all canonical axes		Sum of all canonical axes		Sum of all canonical axes	
	CCA (1)	<i>F</i>	CCA (2)	<i>F</i>	pCCA (1)	<i>F</i>	pCCA (2)	<i>F</i>
Allozymes	.215	1.60*	.092	4.58**	.181	1.32 NS	.055	2.79*
RT3		5.45**						
RT5		3.68*						
RT4		2.67**						
ACC		2.18*						
Glutenins	.618	2.10*	.425	10.32**	.397	1.43 NS	.204	5.15*
RH		10.2**						
RT1		4.10*						
RT3		3.60*						
RT2		3.00*						
DBW		2.40*						
Qualitative	.153	1.11 NS	.079	4.13*	.131	.98 NS	.057	2.99*
	RDA (1)		RDA (2)		pRDA(1)		pRDA (2)	
Phenological	.152	1.76*	.089	7.29**	.093	1.09 NS	.029	2.42*
RH		9.20**						
RT2		8.13**						
Morphological	.168	1.99**	.121	10.28**	.091	1.12 NS	.044	3.76*
ACC		16.79**						
DTN		4.89*						

Note: The five classes of genetic markers are CCA and RDA (1), constrained by environment; CCA and RDA (2), constrained by spatial structure; pCCA and pRDA (1), constrained by environment after removing effect of spatial structure; pCCA and pRDA (2), constrained by spatial structure after removing environmental effect. Test statistics (*F* values) are provided with corresponding level of significance after sequential Bonferroni correction. NS = not significant.

\*  $P < .05$ .

\*\*  $P < .01$ .

loci revealed that observed population-specific parameters of population divergence  $\hat{F}_1$  and  $\hat{F}_2$  lay within or very close to the 95% probability region of these parameters generated under assumption of loci selective neutrality in all six pairwise comparisons (fig. 2 in the online edition of the *American Naturalist*). In other words, none of the 10 loci were detected by the procedure of Vitalis et al. (2001) as experiencing direct or indirect selection effect. For quantitative traits, population subdivision analyses revealed the same pattern as for allozymes: indistinguishable habitat and transect differentiation (13.5% vs. 12.7%) and similar differentiation among classes of microtopographic parameters RT, RH, and ACC (table 2).

#### Canonical Correspondence Analysis and Redundancy Analysis

The CCA showed a significant effect of environmental (topographic) variables on allozyme and glutenin variation, and RDA revealed an effect of topography on morphological and phenological quantitative traits (table 3). Rock type appeared to be the most important predictor

of allozyme variation and the second predictor by importance for glutenin and phenological trait variation. Rock height was the main predictor of glutenin and phenological trait variation. Accessibility to grazing was the main predictor of morphological trait variation. However, when pCCA and pRDA were applied to the same marker data sets with the effect of spatial pattern of sampling (four linear transects) partialled out, a pure environmental (topographic) effect on genetic variation was found to be nonsignificant for all of the five markers.

Partitioning of the total genetic variation into pure (sampling unbiased) environmental variation, variation due to spatial pattern of sampling, and shared (spatially structured, i.e., sampling biased environmental variation) and unexplained (remaining) variation found that the pure environmental (topographic) component was similarly low for all marker types (range: 8.7%–11.3%). For all five markers, the pure environmental component exceeded the other two components but was smaller than the remaining unexplained variation (table 4).

Although the fraction of genetic variation explained by sampling unbiased environmental variation was higher

**Table 4:** Partition of the total variation in five classes of genetic markers into four components: environment, space (spatial pattern), shared, and unexplained by both environment and spatial structure

Genetic markers	Environment	Space	Shared (environment + space)	Unexplained
Allozymes	11.3 (.420 NS)	3.4 (.003*)	2.3	82.9
Glutenins	11.3 (.036 NS)	5.8 (.003*)	6.3	76.5
Qualitative	8.7 (.983 NS)	3.8 (.005*)	1.5	86.0
Phenological	9.3 (.462 NS)	2.9 (.016*)	5.9	81.9
Morphological	9.1 (.396 NS)	4.4 (.013*)	7.7	78.8

Note: Percentages for environment and space are provided with corresponding *P* values after 1,000 permutations by a Monte Carlo permutation test (in parentheses). Significance is reported after sequential Bonferroni correction. NS = not significant.

\*  $P < .05$ .

than that due to spatial pattern of sampling for all three genetic markers, only the effect of the latter was significant. This indicates that the spatial pattern of sampling is responsible for an apparent effect of environment detected by CCA and RDA.

#### *Spatial Structure of Genetic Markers and Environmental Variables*

The autocorrelation analysis performed on the first two principal components showed a generally consistent pattern across the four transects in all marker classes (fig. 3). The correlation of adjacent sampling points was always positive (except one transect for morphological traits) and in most cases was higher than the correlation of points lag >1 apart (fig. 3). For all classes of genetic markers, significant autocorrelation of adjacent sampling points (lag 1) was the most frequently observed across transects. The strength of autocorrelation differed among genetic markers, with quantitative traits showing weakest autocorrelation (fig. 3).

For the set of nominal/ordinal variables, the environmental variation exhibited a significant correlation of adjacent sampling points, which was positive and larger than the correlation of points lag >1 in all transects (fig. 3). No autocorrelation was detected for environmental variation estimated from continuous variables.

There was a significant negative relationship of the first two axes of PCA to the logarithm of the distance separating sampling points in all four transects for allozymes and in three transects of four for glutenins. This relationship was less evident across transects in the other three markers, although several significant cases were observed for each marker (fig. 3).

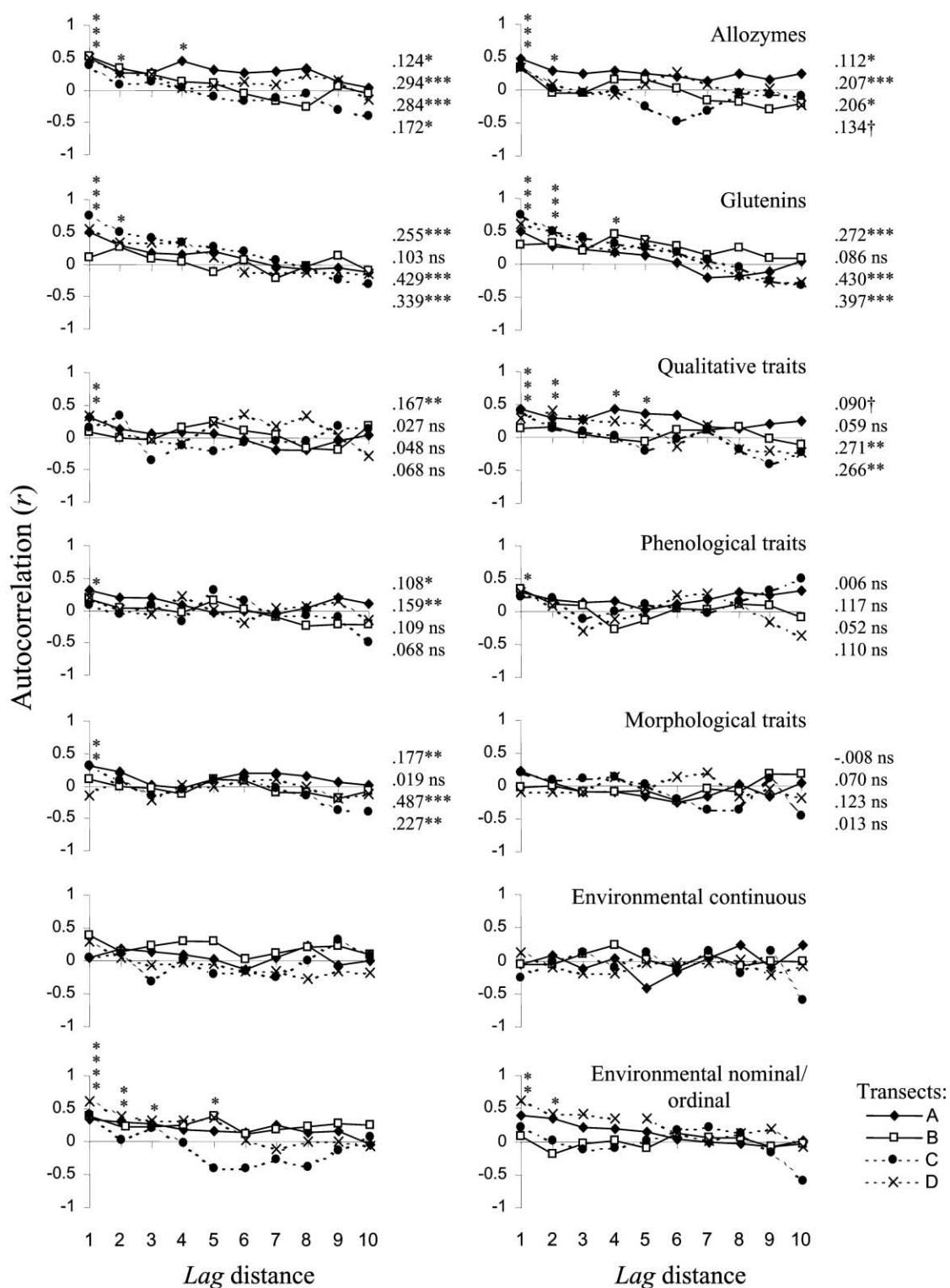
The spatial structure of allozyme markers analyzed with a pairwise correlation coefficient  $I(d)$ , which is an estimate of Wright's coefficient of relationship (Hardy and Veke-

mans 1999), varied among transects and loci (fig. 4). The strongest and most consistent spatial structure across transects was observed for Pgi and Glc. Nine of 10 polymorphic loci exhibited a negative linear relationship of relatedness with the logarithm of distance in at least one transect (table 5). The multilocus estimates of relatedness-distance relationship calculated for each transect were highly significant (table 5).

## Discussion

### *Population Subdivision Analysis*

We undertook a reanalysis of the allozymic data presented in Nevo et al. 1991 and detected a relatively large component of interhabitat variation (16.7%). The interhabitat component of quantitative trait variation was also substantial and similar to that of allozymes (13.5%). This appears to support a conclusion of Nevo et al. (1991) that interhabitat genetic differentiation in emmer wheat is due to selection. However, when we analyzed genetic differentiation among sampling transects, the amount of inter-transect variation did not significantly differ from inter-habitat variation in both allozymes and quantitative traits. This indicates that both pure environmental and pure spatial components are present in analyzed data; that is, habitats are not only environmentally dissimilar but in many cases are also distant from each other. Our data do not allow us to distinguish these components at the spatial scale of habitats because this requires the replication of habitats in space. However, we can attempt to disentangle the above two components at the finer scale of environmental heterogeneity, that is, using original measurements of environmental variability for each sampling point and removing a pure spatial component. The analysis of population structure when groups are the classes of topographic variables that contributed the most to habitat dif-



**Figure 3:** The autocorrelograms for the first two principal components of the PCA applied to allozymes (24.2% and 19.8% of variance explained), glutenins (35.8% and 28.0%), qualitative traits (22.4% and 14.2%), phenological traits (38.0% and 23.7%), morphological traits (29.5% and 19.4%), and two sets of environmental variables: continuous (45.4% and 19.4%) and nominal/ordinal (60.6% and 19.8%). A significant  $r$  value ( $P < .05$ ) for a given transect lag distance is indicated by an asterisk. The negative of the slope of the regression line of the PC axis on the logarithm of distance ( $-b$ ) for sampling transects A–D is shown next to the autocorrelogram, with significance levels indicated by asterisks ( $ns$  = not significant, one asterisk indicates  $P < .05$ , two asterisks indicate  $P < .01$ , and three asterisks indicate  $P < .001$ ).

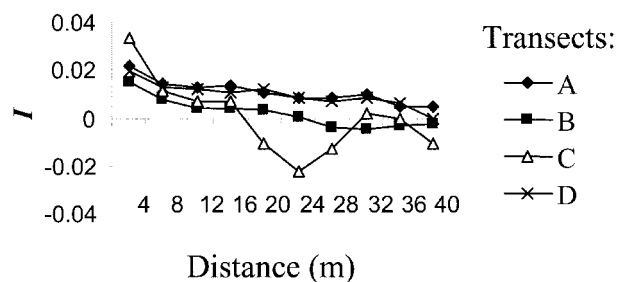


Figure 4: Multilocus allozyme  $I(d)$  values in four sampling transects

ferentiation (e.g., rock type and rock height) revealed the same picture as above: similar amounts of variation among topographic classes and among transects. This, however, is not surprising because, again, different topographic classes are confined to different habitats, which in turn largely coincide with different transects; that is, a spatial component has not been removed. To do the latter, we applied canonical ordination that removed intertransect variation and autocorrelation.

#### Canonical Ordination Analyses

The CCA and RDA detected a significant effect of spatially structured environmental variation on genetic differences between plants for allozymes, glutenins, and quantitative morphological and phenological traits. However, after removing a spatial component of variation in pCCA and pRDA, the relationship of the remaining environmental variation with all five marker types could be explained by chance alone.

This provides a strong argument against any substantial direct effect of measured topographic parameters on fine-scale (meters to tens of meters) population genetic structure except those related to gene flow (see below). This conclusion is reinforced by a consistency of our results across two classes of single-locus genetic markers (allozymes and glutenins) and two classes of markers of polygenic inheritance (phenological and morphological characters). Noy-Meir et al. (1991), analyzing floristic, topographic, and ecological differentiation of habitats within a studied population of emmer wheat, came to the conclusion that microtopographic patterns created by rock outcrops and soil pockets are responsible for the macrotopographically and vegetatively defined habitats. Therefore, it is unlikely (although this possibility cannot be excluded) that habitat-specific selection is responsible for the pattern of genetic variation in *Triticum turgidum diccoides* at a larger spatial scale of topographically defined macrohabitats.

#### Spatial Autocorrelation Analyses

Both the autocorrelation analysis and a comparison of pairwise genetic similarities (data not shown) over increasing distance showed a pattern consistent with one caused by limited gene flow. There was declining positive autocorrelation from lag 1 on in two molecular markers, allozymes and glutenins. In addition, the lag 1 Pearson correlation coefficient in most cases was distinctly larger than succeeding lags in all classes of markers, and the adjacent plants were significantly more similar than plants separated by any larger distance. The pairwise genetic similarity of plants linearly decreased with distance in all markers in at least one transect, with allozymes and glutenins showing the most significant and consistent pattern across transects. Assessment of the spatial genetic structure in allozymes with pairwise correlation coefficients equivalent to Wright's relatedness coefficients also revealed a structure, consistent with the IBD process in most loci and over loci. The loci that exhibited high interhabitat  $\theta_{ST}$  values (Est, Glu, Nadh, Pgi) also had the spatial population structure expected under IBD. Concordance of the genetic structure detected with differently calculated measures of spatial autocorrelation and using different classes of markers than ones expected under IBD suggests that limited gene flow is a primary force determining the genetic structure in this population of emmer wheat.

The results indicate that most seeds are dispersed within a distance of 2–3 m. This is in accordance with the known seed dispersal mechanism of emmer wheat, whose dispersal units (arrow-shaped two-grain spikelets) fall to the ground after spike disarticulation and penetrate into dry soil (Horovitz 1998). Golenberg (1987), using indirect measurement of gene flow by Slatkin's (1985) private-allele regression technique, estimated the mean gene flow distance per generation as about 1.25 m. The topographic autocorrelation exhibited a certain degree of similarity with genetic marker autocorrelation, indicating a similar scale of environmental heterogeneity and seed flow (<4 m, the average distance between adjacent sampling points).

#### Selection or Drift?

Two general scenarios of natural selection effects on genetic population structure are stabilizing and diversifying selection types. Under uniform stabilizing selection, the same phenotype is selected across the range, and the degree of population subdivision is reduced relative to neutral expectation. Under diversifying selection caused by environmental heterogeneity, population subdivision is fostered and exceeds what is expected under neutral expectation. Therefore, discordance between selectively neutral and nonneutral genetic marker population structures is

**Table 5:** Single- and multilocus estimates of the negative of the regression line slope of pairwise correlation coefficients  $I(d)$  on the logarithm of distance ( $-b$ ) in the four sampling transects

Locus	Transect A	Transect B	Transect C	Transect D
Acph	NS	NS	NS	NS
Est	.0121**	.0265***	NS	NS
Gluc	NS	.0399***	.0816***	.0418***
Mdh	NS	NS	NS	.0001***
Nadh	.0125**	NS	NS	.0001***
Pept	NS	NS	NS	NS
Pgi	.0310***	.0331***	.0419***	.0194**
Multilocus weighted mean	.0066***	.0083***	.0173**	.0064***

Note: NS = not significant.

\*\*  $P < .01$ .

\*\*\*  $P < .001$ .

expected in an environment imposing a selection regime strong enough to overcome an effect of gene flow. In contrast, a consistency in population structure revealed by molecular markers and quantitative traits may indicate either the absence of natural selection (at least at the environmental scale studied) or a similar effect of selection on different markers and loci (Lande 1992; Lynch et al. 1999; Whitlock 1999; Merilä and Crnokrak 2001; McKay and Latta 2002). The former stems from the fact that the pattern of population structure exhibited by a marker is independent of the number of loci contributing to that marker (Rogers and Harpending 1983). The latter is hardly possible under random mating because of differential susceptibility of molecular markers and quantitative characters to selection pressures, but it may occur under inbreeding due to high probability of multilocus associations (Brown 1979), low recombination rates, and slow decay of linkage disequilibria (Hedrick 1980; Allard 1989). The latter features are expected to reduce the neutral genetic polymorphism within populations because of lower effective population size (Pollak 1987; Viard et al. 1997), genetic hitchhiking (Hedrick 1980; Schoen et al. 1996), and background selection against deleterious alleles (Charlesworth et al. 1993, 1997) and to promote differentiation at all hierarchical levels due to lower effective population size (Maruyama and Tachida 1992) and limited gene flow (Loveless and Hamrick 1984; Jarne 1995; Hamrick and Godt 1996). Therefore, comparison of  $F_{ST}$  and  $Q_{ST}$  in a selfing species is a more conservative test for neutral selectivity than in an outcrossing species. Indeed, more often the lack of congruence between allozyme and quantitative trait population differentiation was found in the latter (reviewed in Volis et al. 2002b), suggesting that only a strong local selection will cause  $Q_{ST} > F_{ST}$  in a selfer, while drift alone or with moderate selection will result in approximately equal and high values of  $Q_{ST}$  and  $F_{ST}$ . Clegg and Allard (1972) and Hamrick and Allard (1972, 1975)

detected similar population differentiation in allozymes and phenotypic traits reflecting distribution of two types of environments in the predominantly inbreeding *Avena barbata* at both large and small regional scales. On the other hand, a discordant pattern of phenotypic and allozyme variation was reported (Volis et al. 2002b) in the predominantly inbreeding *Hordeum spontaneum* at the large regional scale, and this effect was attributed to the experimentally demonstrated local selection expressed in genotype and population level fitness effects (Volis et al. 2002a, 2003). However, studies conducted at the microgeographical scale (within a population) revealed similar patterns of allozyme and quantitative trait spatial genetic structure consistent with neutral expectation and not consistent with diversifying selection in both outcrossing and selfing species (Bonnin et al. 1996; Hardy et al. 2000).

An explanation of the observed pattern of genetic/phenotypic variation in many studies is an equation with many unknowns, including natural selection effect. In our study, the number of unknowns was reduced by an explicit assumption (to be verified) of habitat-specific (microtopographically defined) selection (Nevo et al. 1991). Under this assumption, the patterns generated by random genetic drift and by natural selection may be indistinguishable due to "linked selection" that has a high chance of occurring in predominant selfers. Under this scenario, genetic hitchhiking will lead to  $Q_{ST} = F_{ST}$  if distribution of both quantitative trait and allozyme variability coincide with distribution of habitats, with abrupt changes in genotypes at habitat transitions. This was not the case in our study. At the same time we found that despite a certain degree of association of genetic and environmental (microtopographic) variation, this relationship was apparent and rose due to similar scale of topographic patchiness and emmer seed dispersal distance. But if the environment was too fine grained with respect to seed dispersal to allow genetic differentiation at the scale of meters (microtopographic

patchiness), why did the patchy genetic differentiation at the larger scale of tens of meters not coincide with the distribution of the habitats? One plausible explanation is that spatial variation at the study site does not impose variation in selection strong enough to lead to local adaptation but physically affects the gene flow (e.g., increase in rockiness reduces number of available soil pockets, thus restricting seed dispersal more than in other habitats). In addition, seed redistribution by seed foragers (Peakall and Beattie 1995; Kalisz et al. 1999) and biotic interactions leading to local extinction with subsequent recolonization (Knowles et al. 1992; McCauley et al. 1995) may be among the major determinants of population genetic structure in wild emmer besides average seed flow and local topography. These potential effects should deserve at least as much attention in future experiment planning as the tests for habitat selection. As for the latter, a test of local adaptation conducted by reciprocal transplanting would be needed to formally reject the assumption of habitat-specific (microtopographically defined) selection in emmer wheat. However, the analysis of existing data provides support to Golenberg's suggestion (Golenberg 1989) that high selfing rates and limited gene flow share the main responsibility for genetic differentiation in wild emmer, with only sporadic (if any) selection pressures. At the same time, our findings do not support an interpretation of observed differences in allozyme frequencies among several macro- or microhabitats as an indication of habitat-specific selection effect in wild emmer (Nevo et al. 1988a, 1988b, 1991).

One conclusion from the present study is that  $Q_{ST}$  versus  $F_{ST}$  comparison may be ineffective in distinguishing the effect of habitat selection from drift in predominantly inbreeding species and at the small scale of tens of meters.

Another important conclusion is that demonstrating an association of variability in a locus or a given genetic marker class with certain environmental variables is necessary but insufficient evidence of their causal relationship as a result of natural selection. Interrelation of several factors or close overlap of their spatial scales may be responsible for apparent selection, thus requiring a deeper insight on the population genetic structure. The latter should include direct or indirect estimation of genetic dispersal distance and use of several classes of genetic markers.

#### Acknowledgments

We would like to thank S. Ezrati for his kind help with data collecting, R. Vitalis for providing Detsel software and guiding, and A. Horovitz for useful comments on earlier versions of the manuscript. We are also grateful to two anonymous referees whose comments greatly improved this article.

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Associate Editor: Philippe Jarne