Theor Appl Genet (2000) 100:985–999

Y.-C. Li · M.S. Röder · T. Fahima · V.M. Kirzhner
A. Beiles · A.B. Korol · E. Nevo

Natural selection causing microsatellite divergence in wild emmer wheat at the ecologically variable microsite at Ammiad, Israel

Received: 9 September 1999 / Accepted: 16 September 1999

Abstract Genetic diversity at 28 microsatellite loci was studied in a natural population of *Triticum dicoccoides* at the Ammiad microsite, north of the Sea of Galilee, Israel. This microsite was subdivided into four major habitats, North, Valley, Ridge and Karst, and further subdivided into nine subhabitats. The units thus defined showed strong and highly significant differentiation in ecological factors; in particular with respect to cover, proximity and height of rocks, and surface soil moisture after early rains. The results showed that allele distributions at microsatellite loci were *nonrandom* and associated with habitats. Significant genetic differentiation and variation in repeat number were found among subpopulations in the four major habitats and nine subhabitats. Habitat-*specific* and -*unique* alleles and linkage disequilibria were observed in the Karst subpopulation. The subpopulations dwelling in drier habitats and subhabitats showed higher genetic diversities at microsatellite loci. These results suggest that natural selection, presumably through aridity stress, acts upon microsatellite divergence predominantly on noncoding sequences, thereby contributing to differences in fitness.

Key words Natural selection · Microecological diversity · Microsatellites · Adaptation · *T. dicoccoides* · Wild emmer wheat

Introduction

Wild emmer wheat, *Triticum dicoccoides*, is the progenitor of all bread wheats (Zohary 1970). Extensive studies have been previously conducted for regional (Israel and Turkey, Nevo and Beiles 1989) and local topographic, edaphic, climatic, and temporal diversities of allozymes (reviewed in Nevo 1988, 1998) and randomly amplified polymorphic DNAs of *T. dicoccoides* (RAPDs, Li et al. 1999). These studies demonstrated that there are significant *nonrandom* genetic differentiation patterns at single- and multi-locus levels, suggesting that these allozymic and RAPD-DNA polymorphisms are at least partly adaptive and differentiated primarily by ecological factors. However, the genetic structures of the noncoding tandem repeated DNA regions in *T. dicoccoides* were little examined at either a macro- or a micro-geographic level.

Microsatellites or simple sequence repeats (SSRs), tandem repeats of short oligonucleotides, are ubiquitously interspersed in eukaryotic genomes particularly in noncoding regions (Tautz and Renz 1984; Kashi et al. 1997; Goldstein and Schlötterer 1999), and show high allelic variation (Thomas and Scott 1993; Becker and Heun 1995; Van Treuren et al. 1997). SSRs have been widely used as DNA markers for identifying cultivars or accessions (Thomas and Scott 1993; Fahima et al. 1998; Plaschke et al. 1995), for genetic mapping (e.g., Becker and Heun 1995; Röder et al. 1998; Peng et al. 1999) and for population genetics and ecology (Saghai-Maroof et al. 1994; Terauchi and Konuma 1994; Todokoro et al. 1995; Awadalla and Ritland 1997; Innan et al. 1997; Van Treuren et al. 1997). Some authors have sought to explain the ubiquitous occurrence of SSRs in term of a functional significance (Kashi et al. 1997; King and Soller 1999; Stallings et al. 1991). But most models of simple repeat evolution, in the absence of close linkage to a selected locus ("genetic hitchhiking", Slatkin 1995), assume selective neutrality (Tachida and Iizuka 1992; Shriver et al. 1993; Valdes et al. 1993; Di Rienzo et al. 1994; Stephan and Kim 1998). In fact, allelic sizes are tightly constrained (Garza et al. 1995; Nauta and Weissing 1996; Dermitzakis et al. 1998). The levels of variation varied in different SSR loci and different populations within species (e.g., Saghai-Maroof et al. 1994;

Communicated by A.F. Linskens

Y.-C. Li · Tzion Fahima · V.M. Kirzhner · A. Beiles · A.B. Korol
E. Nevo (✉)
Institute of Evolution, University of Haifa, Haifa 31905, Israel
e-mail: nevo@research.haifa.ac.il
Tel: +47-972–4-8240448, Fax: +47-972–4-8246554

M.S. Röder
Institute for Plant Genetics and Crop Plant Research,
Corrensstrasse 3, 06466 Gatersleben, Germany

Innan et al. 1997). Mutational biases or selective constraints on allele size may truncate and converge allelic distributions in divergent populations or species (Garza et al. 1995; Amos et al, 1996; Slatkin 1995; Nauta and Weissing 1996; Primmer et al. 1996). Some authors suggested that natural selection controls the level of SSR variation (e.g., Harding et al. 1992; Epplen et al. 1993; Stephan and Cho 1994; Garza et al. 1995; Innan et al. 1997). However, contradictory results were found in different studies.

The present investigation examined the genetic structure at the SSR loci of a natural population of *T. dicoccoides* at the Ammiad microsite, which was vegetationally and topographically subdivided into four major habitats and nine subhabitats. These habitats showed variation in both ecological and floristic features (Anikster and Noy-Meir 1991; Noy-Meir et al. 1991 a, b), as well as phenotypic variation (Anikster et al. 1991) and allozymic variation (Nevo et al. 1991). This paper presents the SSR diversity of *T. dicoccoides* in the 1993 collection from the habitats at the Ammiad microsite in Israel.

Materials and methods

Plant materials

T. dicoccoides (genomic constitution AABB) grows as an annual, highly selfing grass in several steppe-like herbaceous formations in the *Quercus ithaburensis* open park forest belt (Zohary 1973). It grows primarily on basaltic and terra rossa soil types. The investigated site is situated in terra rossa soil in the center of a large area of a *T. dicoccoides* population at Ammiad in the Upper Galilee Mountains, north of the Sea of Galilee, Israel. Sampling *T. dicoccoides* for this study was conducted in 1993 along four transects (A, B, C and D) about 800 m in length at the Ammiad microsite (Fig. 1), as described in Anikster and Noy-Meir (1991), Nevo et al. (1991) and Noy-Meir et al. (1991b). Four major habitats at this microsite were distinguished as 'Valley' (including lower slopes), 'Ridge' (various slopes and aspects), 'North' (a north-facing slope), and "Karst' (with a deeply dissected rock relief) (Fig. 1). At a finer level of resolution, 11 subunits were recognized (only nine subunits were considered here due to incomplete sampling in this study). The local habitat at each sampling point was characterized, to a radius of 50 cm, by measuring topographical position, the micro-relief created by rock out-crops, associated vegetation, soil depth, soil moisture and soil nitrogen (Noy-Meir et al. 1991b). The units thus defined showed strong and highly significant differentiation in ecological factors, in particular cover, proximity and height of rocks, and surface soil moisture after early rains (Table 1). Soil moisture content (% gravimetric) of the 0–5-cm layer in this microsite was measured a few days after the first rains of the season (October–December) during 3 years (Noy-Meir et al. 1991b). The average soil moisture content over the 3 years is presented in Table 1 with a characterization of the rock micro-relief for each habitat and subhabitat. In all years, surface soil moisture was significantly and positively correlated with rock cover, rock height and angle to the top of the nearest rock (Noy-Meir et al. 1991 b). This is because the runoff of rain water from rock surfaces accumulates and saturates in the surface-soil layer nearest to the rock, and that tall rocks reduce evaporation from the soil near rocks by shading and reducing wind speed. The dominant ecological rock micro-relief also modifies grazing pressure and competition. Soil nitrogen shows a different pattern and may be a secondary factor (Noy-Meir et al. 1991b). The Valley was at one extreme (the largest distance to rock from the sampling points, the lowest rock height and angles) with the Karst habitat at the opposite extreme; while North and Ridge habitats were intermediate (Table 1). In the North habitat, rocks were not as close to sampling points but were taller than in the Ridge habitat, while the rock angle was not significantly different between the two habitats. The rocks on the south-facing slope of the Ridge were taller than on its east-facing slope (Table 1). The Karst habitat appears to be the most-favorable environment for plant growth in respect of all the following ecological factors: the highest available soil moisture at both the beginning and the end of the growth season, the highest available nitrogen content, and the lowest grazing pressure. Within the three other major habitats, soil moisture is higher and grazing pressure lower in the Ridge and North than in the Valley, but soil fertility is higher in the Valley (Noy-Meir et al. 1991b). This study included 75 individual plants of wild emmer; 7, 14, 37, and 17 from the four major habitats: the North, Valley, Ridge, and Karst, respectively.

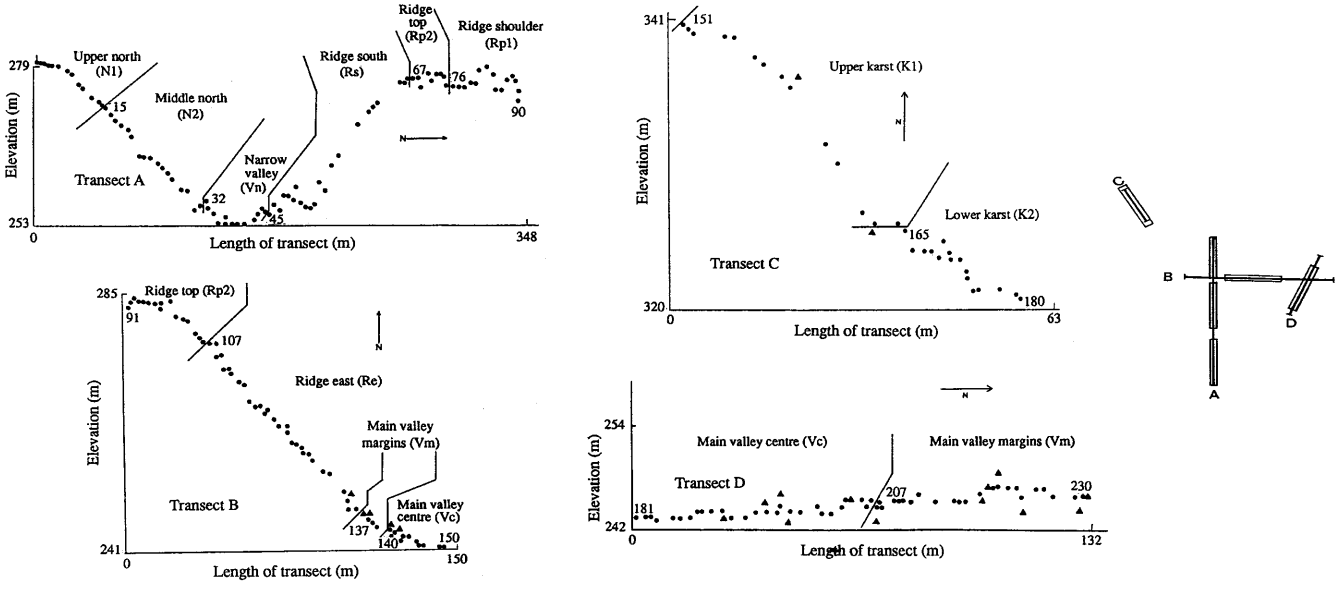

Fig. 1 Topography and diagram of habitats showing sampling points and habitat subunits in transects A, B, C and D at the Ammiad microsite, Israel (from Anikster and Noy-Meir 1991)

Table 1 Ecological characterization of the four vegetationally and topographically defined habitats and 11 subhabitats at Ammiad, Israel. Duncan's multiple range test ($P < 0.05$) and significance of F-ratio of analysis of variance[a]

Habitat units at level		Distance to rock base (HAVDB) (harmonic average, cm)		Height to rock top (AVH) (average, cm)		Angle to rock top (AVA) (average, degrees)		Soil moisture content (%)	
4	11[b]	4	11	4	11	4	11	4	11
North	N1	87a[c]	77ab	27a	20bc	20b	15bcd	26.7b	24.2de
	N2		33bc		34ab		24ab		28.7bc
Valley	Vc	87a	85a	10c	6d	9c	7a	24.2c	24.2de
	Vm		107a		9d		10de		24.0de
	Vn		51b		22bc		13de		25.6bc
Ridge	Re	31c	34bc	22b	19c	22b	20c	27.3b	27.8b
	Rp1		46bc		19c		16bcd		25.9bc
	Rp2		24c		23bc		25ab		26.6bc
	Rs		25c		28ab		24ab		27.3b
Karst	K1	30bc	29c	31a	29ab	26a	21bcd	30.7a	29.9ab
	K2		30c		33a		29a		31.2a
Analysis of variance: $P(F) <$		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[a] The data in this table were extracted from Noy-Meir et al. (1991b)
[b] N_1 = upper north-facing moderate slope, N2 = middle north-facing steep slope; Vc = main valley center, Vn = narrow valley, Vm = valley margin, K_1 = upper Karst, K_2 = lower Karst, Re = ridge, east-facing slope, Rs = ridge, south-facing slope; Rp_1 = ridge, shoulder of plateau; Rp_2 = ridge, top of plateau
[c] Different letters near values means significant difference (Duncan's test)

Analysis of SSR variation

Genomic DNA was extracted from *T. dicoccoides* seedlings by using the method of Junghans and Metzlaff (1990). The SSR primers employed in this study were described by Röder et al. (1995, 1998). Twenty eight dinucleotide SSRs (one for each haploid chromosomal arm: see Table 2) were chosen for the analysis. Table 2 presents the repetitive motif and chromosomal location in Chinese Spring (*T. aestivum*) of each of these SSRs. The procedure used to detect SSR polymorphism followed Fahima et al. (1998). The fragment sizes were calculated using the Fragments Manager (Pharmacia) computer program by comparing with internal size standards, which were added to each lane in the loading buffer. The repeat number of alleles was calculated according to the band sizes and the number of repeat units at the corresponding loci in Chinese Spring (Röder et al. 1995, 1998). At each locus, if the difference among band sizes were ≥ 2 bp (≥ 31 repeat unit), the bands were regarded as different alleles; if not (i.e., a difference of 0–1 bp), they were considered to be the same allele.

Data analysis

T. dicoccoides plants were considered homozygous because of selfing with a very low outcrossing rate (1%, Golenberg 1986). Gene diversity (*He*) and the relative degree of genetic diversity (G_{ST}) in the four major habitats were estimated for each locus according to Nei (1973). Genetic distances among subpopulations in the four habitats were estimated by the allele-sharing distance (D_{AS}, Chakraborty and Jin 1993). The programs *MICROSAT* (Goldstein et al. 1995), *POPGENE* (Yeh et al. 1997) and *STATISTICA* (Statsoft 1996) were used to analyze the data. The χ^2 or Fisher's exact test was used for a homogeneity test of allele frequencies. Since the repeat number at a SSR locus is not normally distributed, a nonparametric method, the Kruskal-Wallis test (K-W) test, was used to analyze the difference in the averages of repeat number and allele number among subpopulations. Levene's test (Statsoft 1996) was used for the homogeneity test of variance in the repeat number at SSR loci. In order to test the effect of random events (e.g. genetic drift and/or founder effect), a permutation test was performed for habitat-specificity or uniqueness of alleles and linkage disequilibria, based on 5000 random shuffling samples.

Results

Distribution of alleles at polymorphic SSR loci

Of the 28 SSR primers, 24 amplified DNA fragments in all samples. Each of 23 markers produced only one band per genotype, and was regarded as one locus. GWM332 amplified two fragments in all samples, which were considered as two loci defined as GWM332a and GWM332b. Four markers (GWM368, GWM408, GWM577 and GWM637) were excluded from the data analysis because of missing data or no amplification products. In total, 25 loci were included in the following genetic analyses. Of these, two loci (GWM601 and GWM361) were monomorphic in all habitats and 23 loci were polymorphic. The allele frequencies for the 23 polymorphic loci appear in the Appendix. The proportions of polymorphic loci were P = 0.76, 0.92, 0.92, and 0.84 in the North, Valley, Ridge, and Karst habitats, respectively (Table 2).

One to twelve alleles were amplified at the 25 loci, and the numbers of alleles differed at all 23 polymorphic loci of the four habitats. The total numbers of alleles over all loci were 62, 87, 118, and 63 in the North, Valley, Ridge, and Karst subpopulations, respectively. The average numbers of alleles per locus were 2.5, 3.5, 4.7, and 2.5 in North, Valley, Ridge, and Karst, respectively (Table 2).

Table 2 Microsatellite DNA markers used in this study: repeat types and chromosomal locations in Chinese Spring, and number of alleles amplified at each locus in the subpopulations from the four major habitats at the Ammiad microsite, Israel

Marker	Repeat type	Chromosomal location	Number of alleles				
			North	Valley	Ridge	Karst	Overall
GWM018	$(CA)_nGA(TA)_k$[a]	1BS	1	2	5	2	6
GWM060	$(CA)_n$	7AS	1	3	4	2	5
GWM095	(AC)	2AS	2	2	3	4	6
GWM099	$(AC)_n$	1AL	1	3	2	1	4
GWM120	$(CT)_n(CA)_k$	2BL	2	4	4	2	4
GWM124	$(CT)_n(GT)_k$imp[b]	1BL	2	5	7	4	7
GWM136	$(CT)_n$	1AS	2	4	7	3	9
GWM162	$(CA)_nAA(CA)_k$	3AL	3	3	4	3	5
GWM169	$(GA)_n$	6AL	4	6	10	3	12
GWM186	$(GA)_n$	5AL	4	6	7	2	9
GWM218	$(CT)_n$	3AS	3	4	5	2	6
GWM219	$(GA)_n$imp	6BL	5	2	5	2	6
GWM251	$(CA)_n$	4BL	2	2	4	1	4
GWM294	$(GA)_nTA(GA)_k$	2AL	1	2	6	3	6
GWM332a	$(GA)_n$	7AL	3	3	3	2	3
GWM332b	$(GA)_n$	7AL	4	5	6	4	9
GWM340	$(GA)_n$	3BL	2	2	3	3	4
GWM361	$(GA)_n$imp	6BS	1	1	1	1	1
GWM389	$(CT)_n(GT)_k$	3BS	3	5	7	3	8
GWM415	$(GA)_n$imp	5AS	3	2	2	2	4
GWM429	$(CT)_n$	2BS	2	2	3	2	3
GWM459	$(GA)_n$	6AS	3	4	5	3	5
GWM537	$(CA)_n(TA)_k$	7BS	3	8	8	5	8
GWM540	$(CT)_n(CC)(CT)_k$	5BS	4	6	6	3	8
GWM601	$(CT)_n$	4AS	1	1	1	1	1
Total			62	87	118	63	143
Mean allele no. per locus			2.5	3.5	4.7	2.5	5.7
Habitat-unique alleles			5	2	22	9	–
Proportion of polymorphic loci			0.76	0.92	0.92	0.84	0.92
Sample size			7	14	37	17	75

[a] *n* and *k*=numbers of repeats
[b] imp = imperfect. GWM368, 408, 577, and 637 were excluded from the list due to no amplifications in most or all samples

The habitats significantly affected the number of SSR alleles [(K-W test), $H_{(3,96)} = 19.555$, $P < 0.001$]. Excluding rare alleles (observed in ≤5 individuals), 19 were found to be karst-*specific* (i.e. predominating in the Karst habitat, χ^2-test, $P < 0.01$–0.00005). Among them, six alleles were Karst-*unique*: $GWM018_{36}$, $GWM060_{17}$, $GWM095_{22}$, $GWM136_{58}$, $GWM162_{21}$ and $GWM218_{26}$. These alleles were found at the six loci with the motifs $(CA)_n$ and $(CT)_n$. The Karst-*unique* alleles were almost fixed in the Karst subpopulation (allele frequency > 0.82, see Appendix). In the Ridge subpopulation, two alleles ($GWM060_{22}$, $GWM136_{67}$) were Ridge-*unique* (see Appendix). The permutation test showed that out of 5000 random shuffling samples, no cases were found with more habitat-specific alleles than the observed. The probability of obtaining the observed habitat-*specific* and -*unique* alleles by chance was < 0.0002. Particularly, in most (> 70%) of the individuals tested from the Karst habitat, alleles were distributed in clusters at all polymorphic loci except GWM136, GWM332b, GWM340 and GWM537 (see Appendix). These results indicated that allele distributions were *nonrandom*, and associated with habitats across the small area of the microsite at Ammiad.

SSR variation in repeat number

The K-W test indicated that the average repeat number (ARN) was significantly different across the four habitats at 16 loci (Table 3). At six loci (GWM099, GWM120, GWM162, GWM219, GWM415 and GWM540), the ARNs were significantly smaller in Karst than in the other subpopulations. However, at the other ten loci, the ARNs in the Karst subpopulation were mostly significantly larger than in the others.

The variances in repeat number were also significantly (Levene's test, $P < 0.05$–0.00005) different among the four subpopulations at most loci (Table 3). The order of the variances among the four subpopulations varied at different loci. In the Karst subpopulation, those loci carrying the largest or smallest ARNs also showed the highest variance, e.g. GWM018, GWM095, GWM218, GWM332b and GWM415. These results suggest that the distributions of repeat number (alleles) varied among the different subpopulations, i.e. they are differentiated according to habitat.

Table 3 Average and variance of repeat number, gene diversity (*He*) and genetic differentiation (G_{ST}) at polymorphic microsatellite loci in *T. dicoccoides* of the four habitats at the Ammiad microsite, Israel

Loci	Average repeat number[a]				K-W[b] Test	Variance				Levene's test	Gene diversity (*He*)				G_{ST}
	North	Valley	Ridge	Karst		North	Valley	Ridge	Karst		North	Valley	Ridge	Karst	
GWM018	21.0	20.8	22.1	33.4	33.92****[c]	0.00	0.18	11.02	34.74	8.28***	0.000	0.335	0.606	0.291	0.433
GWM060	9.0	6.5	14.5	15.2	10.85*	0.00	34.73	9.73	24.82	28.59****	0.000	0.561	0.737	0.208	0.485
GWM095	13.1	14.3	14.2	21.1	52.69****	2.14	0.53	0.58	5.49	4.91**	0.408	0.245	0.156	0.311	0.485
GWM099	17.0	18.9	19.1	7.0	42.43****	0.00	156.59	21.34	0.00	6.93***	0.000	0.520	0.142	0.000	0.665
GWM120	33.3	32.4	32.6	32.1	17.19****	0.24	0.71	0.52	0.24	5.01**	0.408	0.541	0.614	0.111	0.324
GWM124	21.3	33.1	42.1	49.4	22.02****	128.57	283.98	216.68	70.76	9.08****	0.245	0.674	0.814	0.464	0.236
GWM136	50.7	41.7	45.7	56.8	9.65*	736.33	315.90	264.95	12.74	7.80***	0.444	0.612	0.809	0.480	0.249
GWM162	25.4	25.4	26.0	22.2	8.70*	14.95	13.80	11.83	7.94	0.97	0.571	0.571	0.647	0.305	0.257
GWM169	31.0	31.9	32.2	27.8	2.76	44.00	35.76	42.27	7.40	15.44****	0.694	0.776	0.863	0.215	0.227
GWM186	17.0	18.2	16.3	16.6	0.29	96.33	99.57	165.77	55.74	4.43**	0.612	0.745	0.776	0.291	0.238
GWM218	7.7	7.4	7.6	22.1	21.87****	2.91	4.42	20.74	74.74	6.27**	0.571	0.663	0.733	0.291	0.276
GWM219	26.0	32.9	30.1	21.4	36.04****	40.14	0.07	20.19	13.34	10,69****	0.776	0.133	0.676	0.206	0.375
GWM251	24.1	25.1	24.8	26.0	1.16	24.14	4.75	9.29	0.00	6.67***	0.245	0.245	0.468	0.000	0.086
GWM294	16.0	13.9	15.4	17.3	16.19***	0.00	8.90	18.14	7.97	5.00**	0.000	0.459	0.662	0.388	0.405
GWM332a	17.6	18.1	17.9	18.1	1.25	0.95	1.06	0.97	0.06	55.16****	0.653	0.602	0.647	0.111	0.230
GWM332b	25.7	29.2	26.9	45.4	28.57****	7.91	47.41	16.40	64.74	1.87	0.735	0.735	0.722	0.533	0.182
GWM340	30.9	34.2	33.6	32.9	6.14	9.14	4.31	4.08	23.26	6.35***	0.245	0.497	0.495	0.623	0.227
GWM389	27.6	28.4	30.9	31.6	6.50	42.95	34.86	18.71	0.38	7.03***	0.653	0.674	0.642	0.491	0.116
GWM415	23.3	22.8	22.5	19.7	31.38****	0.14	0.18	1.92	2.47	3.49*	0.449	0.337	0.234	0.291	0.382
GWM429	16.4	15.0	11.1	16.9	50.18****	0.29	6.46	10.69	0.11	24.20****	0.490	0.245	0.440	0.208	0.419
GWM459	26.6	27.6	27.2	28.6	20.97****	0.62	1.50	1.34	0.62	2.08	0.449	0.694	0.736	0.398	0.181
GWM537	34.9	33.8	30.4	36.5	4.21	48.48	53.72	77.33	41.13	6.22**	0.571	0.806	0.769	0.711	0.063
GWM540	22.0	22.2	21.4	16.4	35.52****	6.67	5.87	4.06	1.63	2.16	0.694	0.776	0.619	0.215	0.302
Mean											0.397	0.498	0.560	0.286	0.291

[a] The average repeat numbers were 17 and 19 at the monomorphic GWM601 and 361, respectively
[b] K-W test = Kruskal-Wallis one-way analysis of variance.
[c] *, **, ***, ****: $P < 0.05, 0.01, 0.001, 0.0001$, respectively

SSR gene diversity among the four habitats

At each SSR locus, gene diversity was different among the four habitats (Table 3). The highest gene diversity across all loci was observed in the Ridge subpopulation (0.560), followed by Valley (0.498), North (0.397) and Karst (0.286). A Wilcoxon signed-rank test showed that gene diversities were significantly different in four of six pairs of comparisons for the four habitats, except those between North and Karst and between Valley and Ridge (Table 4).

Genetic differentiation and genetic distance at four major habitats

The total gene diversity of a subdivided population (H_T) can be analyzed into the mean gene diversity in a population (H_s), the average ($D_{ST} = H_T$-H_s), and the relative (G_{ST}) degree of gene differentiation among subpopulations (Nei 1973). At all SSR loci except GWM251 and 537, the G_{ST} ranged from 0.12 to 0.67, with an average of 0.291 (Table 3), suggesting that 12–67% of the total gene diversity existed between these close microscale habitats at the SSR loci. The average G_{ST} (0.291) indicated that 29% of the total gene diversity existed among the habitats.

Genetic distances among the four subpopulations were estimated by the allele-sharing distance index (D_{AS}, Chakraborty and Jin 1993, Table 4). Karst is the most remote subpopulation from the three other habitats ($D_{AS} \cong 0.7$). The genetic distances among the other habitats were almost half those of the Karst habitat. The smallest D_{AS} (0.288) was observed between Valley and Ridge.

Table 4 Coefficients of allele-sharing genetic distance (D_{AS}, above the diagonal) and differences[a] of gene diversities (*He*, under the diagonal) among subpopulations of *T. dicoccoides* at the four habitats at the Ammiad microsite, Israel

Subpopulation	North	Valley	Ridge	Karst
North		0.375	0.417	0.707
Valley	–0.101*[b]		0.288	0.670
Ridge	–0.164*	–0.062c		0.678
Karst	0.111c	0.212***	0.275****	

[a] The difference = *He* of subpopulation of the column, *He* of the row. The Wilcoxon signed-rank test was used for pairwise comparison across all loci
[b] *, **, ***, ****: P<0.05, 0.01, 0.001, 0.0001, respectively
[c] P<0.10

Genetic diversity in eight subhabitats

Within the three habitats, the number of alleles (*A*), the proportion of polymorphic loci (P), and the gene diversities (*He*) differed in subpopulations from subhabitats (Table 5). Within the Karst habitat, higher genetic diversity (*A*, P, and *He*) was found in the Upper Karst (K_1) subpopulation than in the Lower Karst (K_2), although the sample size in K_1 (n = 6) was much smaller than in the K_2 subpopulation (n = 11). Within the Valley habitat, genetic diversity in the Narrow Valley (Vn) was higher than in the Valley Center (Vc) subpopulation. Within the Ridge habitat, higher genetic diversities were observed on the south-facing slope (Rs) and the top of plateau (Rp_2) than on the east-facing slope (Re) and the shoulder of the plateau (Rp_1). The North habitat was not subdivided here because of the small sample size. In the nine subhabitats, the gene diversities (*He*) were ordered as follows: Rp_2 (0.474) > Rs (0.453) > Re (0.433) > North (0.397) > Vc (0.396) > Rp_1 (0.384) > K_1 (0.350) > Vn (0.314) > K_2 (0.199). The Wilcoxon signed-rank test showed that within the Ridge subpopulation the gene diversity in Rp_1 was significantly (P < 0.05) less than in Rp_2, Re and Rs; and within Karst the H_e was highly significantly (P < 0.01), higher in the K_1 than in the K_2 subpopulation (Table 5).

Spatial autocorrelation

Spatial autocorrelation analysis tests whether the observed values (here, allele frequencies) at one locality are more similar to near localities than to more distant ones. This analysis can summarize the pattern of geographic variation exhibited by the response surface of any given variable. The autocorrelation coefficients (e.g.

Table 5 Genetic diversity of microsatellites in subpopulations of *T. dicoccoides* at subhabitats[a] at the Ammiad microsite, Israel

Genetic diversity	Karst			Valley			Ridge						North (n = 7)
	K_1 (n = 6)[b]	K_2 (n = 11)	Shared alleles	Vc (n = 5)	Vn (n = 9)	Shared alleles	Re (n = 9)	Rs (n = 9)	Shared alleles	R_{p1} (n = 6)	R_{p2} (n = 13)	Shared alleles	
No. alleles: (*A*)													
Total	51	48	37	51	66	29	64	66	32	59	76	42	62
Mean	2.0	1.9	1.5	2.0	2.6	1.2	2.0	2.6	1.3	2.4	3.0	1.7	2.5
P[c]	0.80	0.76		0.60	0.76		0.76	0.84		0.84	0.88		0.76
H_e[d]	0.350	0.199		0.314	0.396		0.433	0.453		0.384	0.474		0.397

[a] The North habitat was not subdivided because of the small sample size. For abbreviations, see Table 1
[b] n = sample size
[c] P = proportion of polymorphic loci
[d] *He* = gene diversity (Nei 1973)

Table 6 Spatial autocorrelation analysis of allele frequencies at 50 loci of nine subpopulations of *T. dicoccoides* at the Ammiad microsite, Israel. Moran's I coefficients for each allele in three distance classes are given. The expected values of I were – 0.143 for classes 1 and 3, and – 0.125 for class 2.

Locus	Allele[a]	Distance (m)		
		0–200	200–500	500–800
GWM060	2	– 0.16	– 0.11	– 0.06
	9	0.17	– 0.32	– 0.23
GWM120	32	– 0.11	0.00	– 0.36
	33	– 0.23	– 0.14	– 0.34
	34	– 0.18	– 0.15	– 0.07
GWM124	17	– 0.03	– 0.03	– 0.27
	49	– 0.13	– 0.55**[b]	0.00
	50	– 0.09	0.17	– 0.39
GWM162	30	– 0.01	– 0.19	– 0.19
GWM169	24	– 0.30	– 0.32	– 0.10
	25	0.03	– 0.33	– 0.16
	30	– 0.10	– 0.25	– 0.05
	36	– 0.28	0.03	– 0.13
	38	0.01	– 0.15	– 0.21
	39	– 0.13	– 0.09	– 0.15
GWM186	1	– 0.30	– 0.12	0.00
	19	– 0.08	– 0.26	– 0.06
	32	– 0.08	– 0.06	– 0.21
GWM218	3	– 0.26	– 0.02	– 0.07
	4	– 0.26	– 0.09	– 0.07
	8	– 0.27	– 0.16	– 0.21
GWM219	32	– 0.30	– 0.42	0.00
GWM251	13	– 0.14	– 0.46*	– 0.05
	20	– 0.02	– 0.51*	– 0.26
	26	– 0.15	– 0.26	– 0.17
GWM294	10	– 0.02	– 0.28	– 0.06
GWM332a	17	0.02	0.15	– 0.54*
GWM332b	23	– 0.21	– 0.12	– 0.03
	26	– 0.04	– 0.17	– 0.15
	28	– 0.14	– 0.03	– 0.19
GWM340	32	0.16	– 0.50*	– 0.09
GWM389	18	0.07	– 0.28	– 0.16
	30	– 0.22	– 0.12	– 0.05
	31	– 0.18	– 0.25	– 0.13
GWM429	9	– 0.06	0.11	– 0.36
	16	– 0.16	0.15	– 0.30
GWM459	25	– 0.16	– 0.04	– 0.16
	26	0.04	– 0.40	– 0.12
	27	– 0.02	– 0.22	– 0.12
	28	0.05	– 0.32	– 0.14
GWM537	20	– 0.04	– 0.49*	– 0.05
	25	– 0.30	0.02	– 0.08
	34	– 0.23	– 0.12	– 0.11
	37	– 0.34	– 0.08	– 0.04
	38	– 0.12	0.04	– 0.29
	39	– 0.30	0.00	– 0.08
	41	– 0.41	0.02	0.00
GWM540	23	0.01	– 0.26	– 0.11
	24	– 0.08	– 0.08	– 0.18
	25	– 0.16	– 0.12	– 0.07
Mean		– 0.122	– 0.165	– 0.151
SE		0.019	0.026	0.016

[a] This analysis was performed only for the relatively common alleles in the subpopulations

[b] *, **, $P < 0.05$, 0.01, respectively

Moran's I) and their significance levels are a function of the distance between pairs of localities (Sokal and Oden 1978a, b). We computed the Moran's-I autocorrelation coefficient of relatively common alleles across the entire microgeographic range in our study, including the nine subpopulations. The space was partitioned into three distance classes, so that each class contained equal numbers of locality pairs. The results are presented in Table 6.

Average coefficients

All the average autocorrelation coefficients over all tested alleles in all three distance classes were low and non-significant. In each distance class, positive or negative values of low, medium and sometimes high estimates were found for different alleles, suggesting that there is no similar pattern across loci.

Low order: short-distance (0–200 m) autocorrelation

Positive and negative autocorrelations were intermixed across loci. No positive and significant autocorrelations were found. Although all negative autocorrelations were not significant, their abundance suggests the existence of an opposite pattern to the expected highest positive autocorrelations at low distance.

Multiple regression analysis of ecological factors and SSR diversity

A test of the best predictors of *A*, P and *H*e over all loci in the nine subpopulations was conducted by stepwise multiple regression, using these genetic indices as dependent variables and ecological factors as independent variables. The following ecological factors were included in the analysis: distance to rock base (HAVDB), soil moisture content (M) averaged for 3-year measurements, average height to rock top (AVH) and angle to rock top (AVA). The best two variable-predictors of *A*, P and *H*e were HAVDB and M, explaining significantly ($P < 0.01$) 0.86, 0.87 and 0.80 of their variances, respectively (Table 7). The best three predictors including HAVDB, M and AVH could significantly ($P < 0.01$) explain 0.89 and 0.92 of the variances of P and *A* (Table 7). These results suggested that a substantial amount of genetic diversity at the Ammiad microsite is associated with some of the micro-ecological variables.

Discriminant analysis

Stepwise discriminant analyses were conducted to distinguish between the four subpopulations and classify individuals into the four habitats. The program computed three canonical functions based on eight (GWM095, GWM124, GWM162, GWM186, GWM219, GWM332a,

Table 7 Coefficient of multiple regression (R^2) of genetic diversity and micro-ecological variables (as independent variables) in the nine vegetationally and topographically defined subhabitats of *T. dicoccoides* at Ammiad

Step	Genetic diversity					
	P[a]		Number of alleles		*He*[c]	
	Variable[b]	R^2 (n = 9)	Variable	R^2 (n = 9)	Variable	R^2 (n = 9)
1	HAVDB	0.711**[d]	HAVDB	0.203	M	0.196
2	HAVDB, M	0.862**	HAVDB, M	0.873**	M, HAVDB	0.796**
3	HAVDB, M, AVH	0.887**	HAVDB, M, AVH	0.920**	–	–

[a] P = proportion of polymorphic loci
[b] Four ecological variables are included in this multiple regression analysis: HAVDB = distance to rock base (harmonic average, cm), M = soil moisture content (%) averaged 3-year measurements, AVH = average height to rock top. AVA = angle to rock top. All data of these variables were extracted from Noy-Meir et al. (1991b)
[c] *H*e = gene diversity (Nei 1973)
[d] **, $P < 0.01$

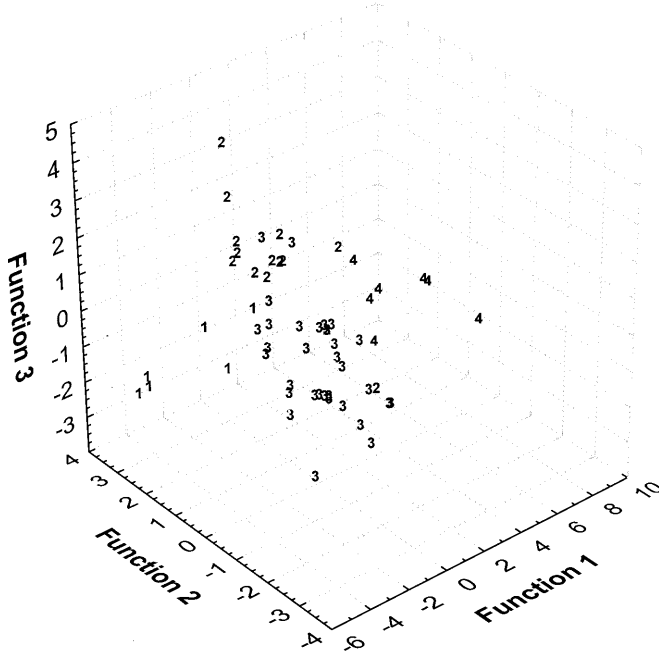

Fig. 2 Scatterplot of 70 plants of *T. dicoccoides* from the four major habitats at the Ammiad microsite in the spaces of canonical discriminant functions one, two and three based on eight polymorphic loci. *1* = North, *2* = Valley, *3* = Ridge, *4* = Karst

GWM389 and GWM429) out of 23 polymorphic loci. The overall multivariate F ratio for differences among the subpopulation centroids was highly significant [$F_{(24, 185)} = 17.08$, $P < 0.00005$]. These functions were sufficient to correctly classify 85% of 70 individuals into their original habitats. Figure 2 shows the plot of these 70 individuals in the space of the three canonical discriminant functions. With respect to five loci (GWM095, GWM124, GWM186, GWM219 and GWM429), 81% of the 70 individuals could be correctly classified into their original habitats, the difference among the subpopulation centroids being highly significant [$F_{(15, 184)} = 23.46$, $P < 0.00005$]. Moreover, even for three loci (GWM095, GWM219 and GWM429), 75% of the 70 individuals could be correctly assigned to their original habitats; the difference among the subpopulation centroids was also highly significant [$F_{(15, 175)} = 33.88$, $P < 0.00005$]. These results suggested that the genetic differentiation was substantial.

Linkage disequilibrium (LD) between SSR loci

In the present study, 8.0%, 12.3%, 13.1%, and 36.0% of the total 1413, 3297, 6259, and 1626 two-allele pairs between loci in North, Valley, Ridge, and Karst respectively displayed significant ($P < 0.05$) LDs (Table 8a). In the four subpopulations, the percentages of significant LDs between loci over all genomes, within genome A, within genome B, between genome A and B, and within chromosomes were significantly ($P < 0.01$–0.00005) larger than the 5% expected by chance. In the Karst subpopulation, the percentage of significant LDs was in most cases significantly (χ^2-test, $P < 0.05$–0.00005) higher than in the other subpopulations (Table 8a). Sample size may affect the significant LDs. However, the sample size at Karst (n = 17) was much smaller than that at Ridge (n = 37) and similar to that in Valley(n = 14). This may suggest that the significant difference in the percentages of LDs do not result from different sample sizes. We found that 94% and 96% of LDs were significantly (at $P < 0.05$ and $P < 0.01$ levels) different between the two extreme opposite Valley and Karst subpopulations; namely, these LDs were habitat-*specific*. Many habitat-*unique* LDs were also found at different loci; some examples are presented in Table 8b. Random drift and founder effects may also affect the LDs. In order to determine the significance of the observed habitat *specificity* or *uniqueness* of LDs, a permutation test was performed. Out of 5000 random permutations, no cases showed more Karst-*specific* or -*unique* LDs, suggesting high significance ($P < 0.0002$) of the observed Karst-*specificity* and -*uniqueness* for LDs. After exclusion of the rare alleles (observed in ≤ 5 individuals in the whole population), the LD for karst-*specificity* or -*uniqueness* remained the same based on the 5000 random permutations. This result suggested that the Karst-*specific* or -*unique* LDs do not arise from chance events, e.g. random drift.

Table 8

a Pairs of significant ($P < 0.05$) linkage disequilibria in the four major subpopulations of *T. dicoccoides* at the Ammiad microsite, Israel

Habitat	North	Valley	Ridge	Karst	Overall
Total pairs	1413	3297	6259	1626	9532
Significant LDs	114	405	819	586	1720
% of the total[a]	8.0**[a]	12.3****	13.1****	36.0****	18.4****
Within genome A					
Total pairs	324	838	1635	428	3088
Significant LDs	20	123	232	195	641
% of the total	6.2	14.7****	21.0****	46.5****	20.8****
Within genome B:					
Total pairs	273	625	1199	293	1496
Significant LDs	21	85	238	73	255
% of the total	7.7	13.6****	19.8****	24.9****	17.0****
Between A and B:					
Total pairs	680	1605	3097	791	4968
Significant LDs	71	185	535	286	824
% of the total	10.4***	11.5****	17.3****	36.2****	16.6***
Within chromosome					
Total pairs	43	103	206	60	216
Significant LDs	4	17	24	22	38
% of the total	9.3	16.5*	11.7*	36.7***	17.8****

[a] χ^2-test for difference of percentage of significant LDs from the 5% expected by chance. *, **, ***, ****: $P < 0.05$, 0.01, 0.0005, 0.00005, respectively

b Habitat-*unique* linkage disequilibrium in the Ammiad population of *T. dicoccoides*, Upper Galilee, Israel

$Locus_{allele}$-$locus_{allele}$	Linkage disequilibrium: D			
	North	Ridge	Valley	Karst
$GWM294_{10}$-$GWM415_{23}$	No[a]	0.040	– 0.138**[b]	0.097***
$GWM294_{10}$-$GWM186_{21}$	No	No	0.092*	– 0.097***
$GWM294_{10}$-$GWM332b_{43}$	No	– 0.025	– 0.128*	0.104***
$GWM218_{4}$-$GWM332b_{66}$	No	No	0.061**	– 0.114*
$GWM415_{23}$-$GWM124_{48}$	No	– 0.051	– 0.056*	0.048*
$GWM186_{1}$-$GWM332b_{66}$	No	No	0.061**	– 0.114**
$GWM186_{21}$-$GWM124_{48}$	No	No	0.061**	– 0.048*
$GWM169_{10}$-$GWM540_{14}$	No	– 0.004	0.061**	– 0.052**
$GWM169_{30}$-$GWM219_{33}$	No	0.073**	– 0.061**	No
$GWM060_{9}$ -$GWM540_{19}$	Fixed	0.063**	– 0.153*	No
$GWM095_{15}$ -$GWM540_{14}$	No	0.028*	No	– 0.052**
$GWM136_{44}$ -$GWM095_{14}$	No	– 0.035	No	0.062***
$GWM332b_{43}$ -$GWM429_{13}$	No	0.080*	0.020	– 0.104****
$GWM124_{48}$ -$GWM540_{14}$	No	0.024*	– 0.005	– 0.052**

[a] No = no linkage disequilibium because at least one allele did not exist
[b] *, **, ***, ****: $P < 0.05$, 0.01, 0.001,0.00005 respectively (χ^2 test)

Table 9 Genome organization based on the 23 polymorphic loci for the subpopulations of *T. dicoccoides* from the four habitats at the Ammiad microsite, Israel

Habitat	Expected variance of K[a]	Observed variance of K	Upper limit of 95%	X(2)[b]
North	4.249	25.876*[c]	8.605	5.091
Valley	4.846	25.288*	8.380	4.219
Ridge	4.579	20.640*	6.638	3.507
Karst	4.193	39.833*	6.968	8.500
Overall	4.606	21.692*	6.060	3.710

[a] K = number of heterozygous loci in two randomly chosen gametes
[b] X(2) = an index of the intensity of multilocus structure
[c] *, $P < 0.05$

The proportions of significant LDs between loci within the genomes A and B, between A and B, and within chromosomes were similar in the Valley and Ridge subpopulations. However, a significant (χ^2 = 15.01, df = 1, $P < 0.0001$) difference was found within genomes A (46.5%) and B (24.9%, Table 8 a) in the Karst subpopulation. In the North subpopulation, more significant LDs were observed between genomes A and B (10.4%) than within genome A (6.2%) and within B (7.7%). These results may suggest that the effects of genetic factors (ge-

nome, linkage itself) on LDs were not significant in the Valley and Ridge subpopulations, but that the genome effect (A vs B) was very strong on LDs in the Karst subpopulation.

Multilocus structure

The observed and expected variances of K, the number of heterozygous loci between two randomly chosen gametes in a (sub)population (Brown et al. 1980), were estimated (Table 9). The difference between the observed and expected variances were significant ($P < 0.05$) in the four subpopulations. The observed variance of K in the Karst subpopulation was the highest (39.83), and apparently larger than those in North (25.88), Valley (25.29), and Ridge (20.64). The standardized index of the intensity of multilocus structure, X(2), was also obviously higher (8.50) in Karst than in the other habitats, suggesting that a higher level of multilocus association exists in Karst than in the other habitats.

Discussion

Genetic divergence and the ecological theater

Aridity stress may be the major discriminant factor for genetic diversity in Ammiad (Nevo et al. 1991). Specifically, rock factors, both directly (through soil moisture of the early growing season) and indirectly (through the associated vegetation), might contribute to the ecological factors within the population of wild emmer at the Ammiad microsite (Noy-Meir et al. 1991b). Phenotypic variation of wild emmer at Ammiad indicated that both qualitative and quantitative traits of wild emmer varied *nonrandomly* among subpopulations from these habitats, suggesting an adaptation to the habitats (Anikster et al. 1991).

The four major habitats showed highly significant differences in rock micro-relief (Noy-Meir et al. 1991b). The Karst habitat is the ecologically most distinct subunit of this study, with characteristic rock features, soil moisture regime and vegetation (Noy-Meir et al. 1991b). The Karst habitat consists of heterogeneous micro-niches that differ in soil-moisture content during the growing season, depending on their distance from rock walls. The Upper Karst subhabitat was drier and with deeper soil than in the Lower Karst subhabitat (Noy-Meir et al. 1991b). Nevo et al. (1991) found that the highest allozymic gene diversity was in the Karst subpopulation among the four major habitats and, within the Karst habitat, a relatively higher gene diversity was found in the Lower Karst than in the Upper Karst subpopulation. Genetic diversity is assumed to parallel habitat heterogeneity, corroborating the niche-width genetic diversity (Van Valen 1965; Nevo 1988), which visualizes a positive correlation between niche width and the level of genetic diversity.

In our study, genetic diversity (*A*, P, and *H*e) of the SSRs was lower in Karst than in the Valley and Ridge populations. Although the North subpopulation had the smallest size ($n = 7$), its *H*e (essentially: a sample-independent index) was larger than that of the Karst subpopulation, while the two sample-dependent indices (P and *A*) were smaller in the North than in Karst. The highest genetic diversity observed in the Ridge subpopulation may be due to its larger sample size ($n = 37$). However, the higher genetic diversity in the Valley than in the Karst subpopulation cannot be explained by sample sizes ($n = 14$, 17 in Valley and Karst, respectively). Within the Ridge habitat, the south-facing slope (Rs) was drier than the east-facing slope (Re, Noy-Meir et al. 1991b). More alleles and more polymorphic SSR loci were observed in the drier Rs than in the Re subpopulation with the same sample size ($n = 9$). Within the Karst habitat, the relatively drier Upper Karst subpopulation showed more alleles, a higher proportion of polymorphic loci and an almost double *H*e (K_1, $n = 6$; *H*e = 0.350) than the adjacent and relatively wetter Lower Karst subpopulation (K_2, $n = 11$; *H*e = 0.199). The same pattern was also shown by allozymic diversity in the same 1993 sample set (*H*e = 0.052, 0.035 for the K_1 and K_2, respectively). These results consistently demonstrate that the subpopulations dwelling in drier habitats or subhabitats tend to have higher genetic diversities at SSR loci, presumably suggesting an adaptation to water stress. Environmental stresses may accelerate replication errors (Jackson et al. 1998) and recombination intermediates (Korol et al. 1994; Afzal et al. 1995), or decrease the ability of DNA mismatch-repair mechanisms (Radman et al. 1995; Brentnall et al. 1996; Jackson et al. 1998) so as to increase SSR diversity. Ecological stress could also select the favorable alleles or eliminate deleterious mutants, thereby changing the level and pattern of SSR diversity (Hartl and Clark 1997).

Distinct phenotypic divergence of *T. dicoccoides* was found in the deeply creviced Lower Karst (K_2) subhabitat (Anikster et al. 1991). However, we did not find any *specific* or *unique* alleles at any SSR loci assayed for the K_2 subpopulation. By contrast, we did find some *specific* or *unique* alleles distributed across the whole Karst subpopulation at some SSR loci (e.g. GWM018, GWM060, GWM095, GWM162 and GWM218). In the K_2 subpopulation, alleles were fixed, or almost fixed, at 22 polymorphic SSR loci with the exception of GWM340 and GWM537 (data not shown). Similar results were also found in the protein-coding (allozymic) regions (data not shown). Gene flow may not be the reason for the observed allele distributions at Lower and Upper Karst, since it can not create differential pattern. The pattern may suggest that the *unique* ecological forces in the Karst habitat may eliminate or limit mutation, so as to maintain the Karst-*unique* or -*specific* alleles.

Multiple regression analysis indicated that the observed SSR diversities among the subpopulations from the nine subhabitats were significantly associated with

micro-ecological factors, including rock-relief and soil-moisture.

Functional significance of the SSRs

The functional significance of SSRs may explain the genetic pattern in the Karst habitat. The most Karst-*specific* and -*unique* alleles were found at $(GA)_n$ and $(CA)_n$, $(CT)_n$ SSR loci, respectively. The results may suggest that these tandem repeated sequences would have some biologically functional regulatory role in helping plants to adapt to their environment. Although a general function for SSR loci has not been clear, apparently not all SSRs represent pure evolutionary junk (Epplen et al. 1993); certain functions have been observed for some SSRs and these could provide a basis for selection on particular loci (reviewed in Kashi et al. 1997; King and Soller 1999). Previous experiments have already shown that $(TC)_n$, $(GA)_n$, and $(TA)_n$ control the transcriptional activity of the genes *Ultrabithorax, hsp26*, and *actin 5 C,* respectively, in *Drosophila*, and that $(TG)_n$ influences the activity of transcription in the *prolactin* gene of the rat (reviewed in Kashi et al. 1997). The presence of $(TA)_n$ as a part of many regulatory signals (e.g. TATA box, transcription terminators) may help to avoid inappropriate binding of regulatory factors (Karlin et al. 1998). SSRs are functionally integrated into the genome, so that changes in tract length can exert a quantitative regulatory effect on gene transcription activity (King and Soller 1999). SSR alleles undergoing changes in length can nevertheless retain their characteristic mutability. Consequently, selection for favorable alleles would indirectly select the mutability function by which those alleles arise. Conversely, SSR alleles can undergo site-specific modification of their mutability, for example by mutation that alters the purity of motif repetition, while retaining their characteristic effect on phenotype and fitness. Indirect selection can thereby shape the degree of mutability for any gene that includes a functional SSR (King and Soller 1999).

Potential selection scenario of SSRs in *T. dicoccoides*

Habitat-*specificity* was found at both single- and two-locus levels in different habitats, in particular in the Karst subpopulation, suggesting that natural selection may act upon SSR variation. Although *T. dicoccoides* is a selfing plant with a low rate of outcrossing (1%, Golenberg 1986), even a little outcrossing in wild cereals (Brown et al. 1978) can provide raw material for clonal selection, particularly in a widespread population, such as that of Ammiad, involving thousands of wild emmer individual plants. In the present study, more significant linkage disequilibria than the 5% expected by chance were found in the North, Valley, Ridge, and Karst subpopulations. All the percentages of significant linkage disequilibria were higher than that obtained for the outcrossing species honeybee (6.4%, Estoup et al. 1995), and were also higher than that observed in a selfing plant *Arabidopsis thaliana* (12.1%, Innan et al. 1997) except in the case of the North subpopulation. In *T. dicoccoides* at the Ammiad microsite, habitat significantly affected the percentage of significant linkage disequilibria. The Karst subpopulation showed the largest amount of linkage disequilibria and more Karst-*specific* or -*unique* linkage disequilibria. The permutation test suggested that the observed Karst *uniqueness* or *specificity* of linkage disequilibria were not caused by random genetic drift. The *uniqueness* or *specificity* of two-locus associations may be an adaptation to the Karst microenvironment. In the study of the selfing plant *A. thaliana*, Innan et al. (1997) suggested that there was no reason to reject some form of balancing selection on SSRs, although conventional population genetic tests were not applied to see if there was any deviation from neutrality because of the homozygosity of individuals of *A. thaliana*, as in the case of *T. dicoccoides* investigated in this study. Stephan and Cho (1994) built a simulation evolutionary model of tandem-repetitive DNA according to previous experimental data. In this model, natural selection acts upon SSRs as a mechanism for controlling the length of a nucleotide string. SSRs themselves may be either targets of selection, or markers of selected haplotypes. The possibility of SSRs being located in a selected haplotype cannot be excluded, especially because wheat is a selfer.

In an inbreeder such as wild emmer, the neutral model would predict a random distribution of parent plants, each propagating itself in its vicinity and creating the well-known neighbourhood effect. It is important to avoid the pitfalls of neighbouring effects in the attempt to separate random (founder effect) from nonrandom (niche structure) factors. Patches of given genotypes are expected to develop in plant populations when gene dispersal is limited (e.g. Turner et al. 1982). A spurious correlation of gene frequency with the habitat units could easily be observed in a limited number of samples. The pattern of an inbreeder would involve the entire genotype and not just individual, separate alleles. While a neutral model can account for different alleles in different clusters, it cannot generate any systematic differential patterning of genotype clusters distinguishing between habitats and years. It is noteworthy that under a neutral model only factors such as population size and isolation by distance without selection can generate differential patterns of genotypes and alleles. The neutral expectation seems not to meet with the genetic structure at the Ammiad microsite. The negation of the neutral model is based on the following points:

(1) Population size; the population size of wild emmer in the Ammiad is large involving many thousands of individuals and does not represent a Wrightian small population of $n = 10–100$.
(2) Vegetation; both plant association and floristic patterns differ in the four major habitats (Noy-Meir et al. 1991b). Therefore, habitat differentiation of SSRs is

unlikely to be random. Likewise, phenotypic variation (Anikster et al. 1991) and allozymic variation (Nevo et al. 1991) in wild emmer also varies among habitats.

(3) Temporal variation; the observed genetic structure of allozymes and SSRs in the 1993 collection of wild emmer in this study is inconsistent with that of allozymes observed in the 1984–1988 collections by Nevo et al. (1991). Temporal variation is unlikely under a neutral model, since the neutral model does not expect such large changes in large population.

As mentioned above, gene flow may not be the reason for the observed structure, because it does not cause differentiation. The permutation test ruled out the possibility of a stochastic effect. Thus, neighborhood effect and gene flow could not explain the habitat- and subhabitat-structures; natural selection appeared to be the primary effect for the observed microgeographic structure at the Ammiad microsite.

Spatial autocorrelation

The autocorrelation analysis predicts that, starting from the first ones migration will cause a high positive correlation (similarity) in neighboring localities. Migration is expected to result in a similarity between loci and alleles. Our results did not meet this expectation. In contrast, SSR loci of wild emmer wheat differed drastically in their autocorrelation pattern between loci. Our SSR data was structured, and so negated randomness. Environmental selection is also partly autocorrelated and affects loci differentially, as indicated by our data. We found not only variation between loci, but also a positive correlation in distant groups and not necessarily in the first one.

SSR divergence in other microsites, Yehudiyya and Tabigha, of *T. dicoccoides*

Edaphic and microclimatic SSR diversities were also found in our two other populations of *T. dicoccoides* from two microsites, Tabigha and Yehudiyya, near the Sea of Galilee, Israel (Li et al. 1999). The Tabigha microsite included two adjacent niches on terra rossa and basalt soils. The Yehudiyya microsite involved two microclimatic niches (the shady and sunny) distributed mosaically among trees. Soil- and shade-*unique* or -*specific* alleles and linkage disequilibria were found in the two microsites. This proved that edaphic and microclimatic selection appears to be the primary cause for these microniche structures of *T. dicoccoides* (Li et al. 1999).

Conclusions and prospects

In conclusion, SSR allele distributions in Ammiad were nonrandom and associated with the habitats of *T. dicoccoides*. Other explanatory models (stochastic, founder effect, etc.) are unlikely to explain the patterns obtained in this and other studies on the microscale divergence of SSRs. Natural selection appears to be the most-likely explanatory model. Natural selection may act upon SSR variation at both single- and multi-locus levels. SSRs themselves may be either targets of selection or markers of selected haplotypes. The possibility of SSRs being located in a selected haplotype cannot be excluded, especially because wheat is a selfer. SSRs may be genetic agents to achieve fast adjustment of genetic activity in spatially stressful and heterogeneous environments.

Acknowledgments We thank Dr. V. Korzun and Ms. K. Wendehake for their excellent assistance, and the Graduate School, University of Haifa, for financial support for this study. The work was also supported by the Israel Discount Bank Chair of Evolutionary Biology and the Ancell-Teicher Research Foundation for Genetics and Molecular Evolution. The SSR analysis was conducted in Germany at the laboratory of the Institute for Plant Genetics and Crop Plant Research. We also Thank Dr. A. Beharav for performing the spatial autocorrelation analysis. This research is part of the PhD research project of the first author.

References

Afzal V, Feeney L, Thomas GH, Volpe JP, Cleaver JE (1995) Sister chromatid exchanges in cells defective in mismatch, post-replication and excision repair. Mutagenesis 10: 457–462

Amos W, Sawcer SJ, Feaker RW, Rubinstein DC (1996) Microsatellites show mutational bias and heterozygote instability. Nature Genet 13: 390–391

Anikster Y, Noy-Meir I (1991) Studies of a wild wheat population at Ammiad, Israel. Israel J Bot 40: 351–362

Anikster Y, Eshel A, Ezrati S, Horovitz A (1991) Patterns of phenotypic variation in wild tetraploid wheat at Ammiad. Israel J Bot 40: 397–418

Awadalla P, Ritland K (1997) Microsatellite variation and evolution in the *Mimulus guttatus* species complex with a contrasting mating system. Mol Biol Evol 14: 1023–1034

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27: 835–845

Brentnall TA, Crispin DA, Bronner MP, Cherian SP, Hueffed M, et al. (1996) Microsatellite instability in non-neoplastic mucosa from patients with chronic ulcerative colitis. Cancer Res 56: 1237–1240

Brown AHD, Zohary D, Nevo E (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. Heredity 41: 49–62.

Brown AHD, Feldman MW, Nevo E (1980) Multilocus structure of natural population of *Hordeum spontaneum*. Genetics 96: 523–536

Chakraborty R, Jin L (1993) A unified approach to study hypervariable polymorphisms: statistical considerations for determining relatedness and population distance. In: Pena DJ, Chakraborty R, Epplen JT, Jeffrey AJ (eds) DNA fingerprinting: state of the science. Birkhäuser Verlag, Basel, pp 153–175

Dermitzakis ET, Clark AG, Batargias C, Magoulas A, Zouros E (1998) Negative covariance suggests mutation bias in a two-locus microsatellite system in the fish *Sparus aurata*. Genetics 150: 1567–1575

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, et al. (1994) Mutation processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA 91: 3166–3170

Epplen C, Melmer G, Siedlaczck I, Schwiger FW, Mäueler W et al. (1993) On the essence of "meaningless" simple repetitive DNA in eukaryote genomes. In: Pena DJ, Chakraborty R, Epplen JT, Jeffrey AJ (eds) DNA fingerprinting: state of the science, Birkhäuser Verlag, Basel, pp 29–35

Estoup A, Garnery L, Solignac M, Cornuet JM (1995) Microsatellite variation in honey bee (*Apis mellifera L.)* populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics 140: 679–695

Fahima T, Röder MS, Grama M, Nevo E (1998) Microsatellite DNA polymorphism and divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96: 187–195

Garza JC, Slatkin M, Freimer NB (1995) Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. Mol Biol Evol 12: 594–603

Golenberg EM (1986) Multilocus structure in plant populations: population and genetic dynamics of *Triticum dicoccoides*. PhD thesis. State University of New York at Stony Brook, New York

Goldstein DB, Schlötterer C (1999) Microsatellites: evolution and applications. Oxford University Press, London

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463–471

Harding RM, Boyce AJ, Clegg JB (1992) The evolution of tandemly repetitive DNA: recombination rules. Genetics 132: 847–859

Hartl DL, Clark AG (1997) Principles of population genetics (3rd edn.). Sinauer Associates, Sunderland, Massachusetts

Innan H, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in natural populations of wild plant *Arabidopsis thaliana*. Genetics 146: 1441–1452

Jackson AL, Chen R, Loeb LA (1998) Induction of microsatellite instability by oxidative DNA damage. Proc Natl Acad Sci USA 95: 12468–12473

Junghans H, Metzlaff M (1990) A simple and rapid method for the preparation of total DNA. Biotechniques 8: 176

Karlin A, Campbell AM, Mrázek J (1998) Comparative DNA analysis across diverse genomes. Annu Rev Genet 32: 185–225

Kashi Y, King D, Soller M (1997) Simple sequence repeats as a source of quantitative genetic variation. Trends Genet 13: 74–78

King DG, Soller M (1999) Variation and fidelity: the evolution of simple sequence repeats as functional elements in adjustable genes. In: Wasser SP (ed) Evolutionary theory and processes: modern perspectives. Papers in honor of Eviatar Nevo. Kluwer, The Netherlands, pp 65–82

Korol AB, Preygel IA, Preygel SI (1994) Recombination variability and evolution. Chapman and Hall. London

Li YC, Fahima T, Beiles A, Korol AB, Nevo E (1999a) Microclimatic stress differentiation in wild emmer wheat, *Triticum dicoccoides*. Theor Appl Genet 98: 873–883

Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143: 1021–1032

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321–3323

Nevo E (1988) Genetic diversity in nature: patterns and theory. Evol Biol 23: 217–246

Nevo E (1998) Molecular evolution and ecological stress at global, regional and local scales: the Israeli perspective. J Exp Zool 282: 95–119

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

Nevo E, Noy-Meir I, Beiles A, Krugman T, Agami M (1991) Natural selection of allozyme polymorphisms: micro-geographical spatial and temporal ecological differentiations in wild emmer wheat. Israel J Bot 40: 419–450

Noy-Meir I, Agami M, Anikster Y (1991a) Changes in the population density of wild emmer wheat (*Triticum turgidum* var. *dicoccoides*) in a Mediterranean grassland. Israel J Bot 40: 385–395

Noy-Meir I, Agami M, Cohen E, Anikster Y (1991b) Floristic and ecological differentiation of habitats within a wild wheat population at Ammiad. Israel J Bot 40: 363–384

Peng JH, Fahima T, Röder MS, Li YC, Dahan A, Grama A, Ronin YI, Korol AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98: 862–872

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001–1007

Primmer CR, Ellegren H, Saino N, Moller AP (1996) Directional evolution in germline microsatellite mutations. Nature Genet 13: 391–393

Radman M, Matic I, Hallida Y, Taddei F (1995) Editing DNA replication and recombination by mismatch repair: from bacterial genetics to mechanisms of predisposition to cancer in humans. Phil Trans R Soc Lond B Biol Sci 347: 97–103

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246: 327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149: 2007–2023

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allad W (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91: 5466–5470

Shriver MD, Jin L, Chakraborty R, Boerwinkle E (1993) VNTR allele frequency distributions under the stepwise mutation model: a computer-simulation approach. Genetics 134: 983–993

Slatkin M (1995) Hitchhiking and associative overdominance at a microsatellite locus. Mol Biol Evol 12: 473–480

Sokal RR, Oden NL (1978a) Spatial autocorrelation in biology. I. Methodology. Biol J Linn Soc 10: 199–228

Sokal RR, Oden NL (1978b) Spatial autocorrelation in biology. II. Some biological implications and four implications of evolutionary and ecological interest. Biol J Linn Soc 10: 229–249

Stallings RL, Ford AF, Nelson D, Torney DC, Hildebrand CE, Moyzis RK (1991) Evolution and distribution of (GT)*n* repetitive sequences in mammalian genomes. Genomics 10:807–815

Statsoft (1996) STATISTICA for Windows [computer program manual]. Tulsa, Oklahoma: Statsoft, Inc, 2300 East 14th Street, Tulsa, Oklahoma 74104, USA

Stephan W, Cho S (1994) Possible role of natural selection in the formation of tandem repetitive noncoding DNA. Genetics 136: 333–341

Stephan W, Kim Y (1998) Persistence of microsatellite arrays in finite populations. Mol Biol Evol 15: 1332–1336

Tachida H, Iizuka M (1992) Persistence of repeated sequences that evolve by replication slippage. Genetics 131: 471–478

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12: 4127–4138

Terauchi R, Konuma A (1994) Microsatellite polymorphism in *Dioscorea tokoro*, a wild yam species. Genome 37: 794–801

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor Appl Genet 86: 985–990

Todokoro S, Terauchi R, Kawano S (1995) Microsatellite polymorphisms in natural populations of *Arabidopsis thaliana* in Japan. Jpn J Genet 70: 543–554

Turner ME, Stephens JC, Anderson WW (1982) Homozygosity and patch structure in plant populations as a result of nearest-neighbor pollination. Proc Natl Acad Sci USA 79: 203–207

Valdes AM, Slatkin M, Freimer NB (1993) Allele frequencies at microsatellite loci: The stepwise mutation model revisited. Genetics 133: 737–749

Van Treuren, R, Kuittinen H, Kärkkäinene K, Baena-Gonzalez E, Savolainen O (1997) Evolution of microsatellites in *Arabis petraea* and *Arabis lyrata*, outcrossing relatives of *Arabidopsis thaliana*. Mol Biol Evol 14: 220–229

Van Valen L (1965) Morphological variation and width of ecological niche. Am Nat 99: 377–390

Yeh FC, Yang RC, Boyle T, Ye ZH, Mao JX (1997) POPGENE**,** the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada

Zohary D (1970) Centers of diversity and centers of origin. In: Frankel OH, Bennet E (eds) Genetic resources in plants – their exploration and conservation. Blackwell, Oxford, pp 33–42

Zohary M (1973) Geobotanical foundations to the Middle East, vols 1 and 2. Fischer, Stuttgart/Swets and Zeitlinger, Amsterdam

Appendix

Allele size and frequency[a] of polymorphic microsatellite loci in four subpopulations of *T. dicoccoides* at the four different habitats at the Ammiad microsite, Israel

Locus	Allele size (repeat number)	Allele frequency			
		North (n = 7)	Valley (n = 14)	Ridge (n = 37)	Karst (n = 17)
GWM018	20		0.21	0.24	
	21	1.00	0.79	0.57	0.18
	22			0.08	
	30			0.05	
	32			0.05	
	36				**0.82**
GWM060	2		0.50	0.30	0.12
	9	1.00	0.43	0.15	
	17				**0.88**
	22			**0.27**	
	23		0.07	0.27	
GWM095	11	0.29			
	14	0.71	0.86	0.92	0.06
	16		0.14	0.03	0.06
	17			0.06	
	21				0.06
	22				**0.82**
GWM099	7		0.21		1.00
	17	1.00	0.64	0.92	
	34			0.08	
	47		0.14		
GWM120	31		0.07	0.03	
	32		0.64	0.43	0.94
	33	0.71	0.14	0.43	
	34	0.29	0.14	0.11	0.06
GWM124	17	0.86	0.50	0.24	0.06
	46		0.07	0.08	
	47	0.14	0.14	0.11	
	49		0.07	0.16	0.06
	50			0.08	0.18
	52		0.21	0.27	0.71
	59			0.05	
GWM136	34	0.67	0.14	0.13	
	35		0.57	0.33	
	36		0.14	0.13	
	43			0.08	
	44			0.08	0.07
	57				0.27
	58				**0.67**
	67			**0.17**	
	82	0.33	0.14	0.08	
GWM162	20	0.29	0.29	0.19	
	21				**0.82**
	23			0.08	
	27	0.57	0.57	0.51	0.12
	30	0.14	0.14	0.22	0.06
GWM169	23			0.08	
	24	0.14		0.05	
	25	0.14	0.36	0.19	
	27	0.29			0.88
	30		0.14	0.14	0.06
	31			0.03	
	33			0.03	
	36		0.07	0.11	
	37		0.21		
	38	0.43	0.14	0.22	0.06
	39		0.07	0.08	
	42			0.08	
GWM186	1	0.14	0.14	0.35	0.18
	15	0.57	0.43	0.19	
	19			0.05	
	20		0.14		0.82
	22			0.05	
	26	0.14			
	29		0.07	0.11	
	31		0.14	0.03	
	32	0.15	0.07	0.22	
GWM218	3		0.07	0.24	
	4	0.14	0.14	0.14	0.18
	8	0.57	0.43	0.14	
	9	0.29	0.36	0.41	
	20			0.08	
	26				**0.82**
GWM219	18	0.14		0.03	
	21	0.14		0.11	0.88
	23	0.29			
	26		0.07	0.11	
	32	0.14		0.30	0.12
	33	0.29	0.93	0.43	
GWM251	13	0.14		0.03	
	20		0.14	0.16	
	26	0.86	0.86	0.70	1.00
	27			0.11	
GWM294	10		0.36	0.30	0.12
	16	1.00	0.64	0.49	
	18			0.05	0.76
	20			0.03	0.12
	22			0.08	
	24			0.05	
GWM332a	17	0.43	0.36	0.25	
	18	0.29	0.14	0.31	0.94
	19	0.29	0.50	0.44	0.06
GWM332b	23	0.29		0.06	0.06
	24	0.29	0.36	0.44	0.12
	26		0.21	0.11	
	27		0.07	0.11	
	28	0.14		0.06	
	29	0.29			
	34		0.29	0.22	
	48		0.07		0.65
	49				0.18
GWM340	24	0.14			0.18
	32	0.86	0.46	0.61	0.35
	36		0.54	0.36	
	37			0.03	0.47

Appendix (continued)

Appendix (continued)

Locus	Allele size (repeat number)	Allele frequency			
		North (n = 7)	Valley (n = 14)	Ridge (n = 37)	Karst (n = 17)
GWM389	15			0.03	
	18	0.29	0.21	0.03	
	26		0.07	0.03	
	30			0.08	0.06
	31	0.43	0.50	0.56	0.29
	32	0.29	0.07	0.17	0.65
	34		0.14		
	38			0.11	
GWM415	19			0.14	0.82
	22	0.14	0.21		
	23	0.71	0.79	0.87	0.18
	26	0.14			
GWM429	9		0.14	0.69	
	16	0.57	0.86	0.28	0.88
	17	0.43		0.03	0.12
GWM459	25	0.14		0.08	0.06
	26	0.14	0.21	0.17	0.19
	27	0.72	0.36	0.42	
	28		0.07	0.17	0.75
	29		0.36	0.17	
GWM537	20	0.14	0.14	0.33	0.13
	25		0.07	0.11	
	32		0.14	0.06	
	34	0.29	0.07	0.03	
	37		0.07	0.08	0.13
	38		0.07	0.03	0.25
	39	0.57	0.36	0.31	0.44
	41		0.07	0.06	0.06
GWM540	16		0.07	0.03	0.88
	17	0.14		0.06	
	18			0.06	0.06
	21	0.29	0.36	0.20	0.06
	22		0.14	0.57	
	23	0.14	0.07		
	24	0.43	0.14		
	25		0.21	0.09	

[a] The habitat-*unique* alleles were bolded and underlined.