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Climatic effects on microsatellite diversity in wild emmer wheat (*Triticum dicoccoides*) at the Yehudiyya microsite, Israel

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Microsatellite (SSR) diversity at 28 loci comprising seven types of tandem dinucleotide repeated motifs was analyzed in 105 individual plants of wild emmer wheat, *Triticum dicoccoides*, from a microsite in Yehudiyya, northeast of the Sea of Galilee, Israel. The study area was less than 1000 m^2 and involved 12 paired plots distributed in a mosaic pattern. Each experiment involved very close (a few meters apart), but sharply divergent, microclimatic niches in the open park forest of Tabor oak: (1) sun, between trees, and (2) shade, under tree canopy. Significant microclimatic divergence characterized many loci displaying asymmetric and *non-random* distribution of repeat numbers. Niche-*specific* and niche-*unique* alleles and linkage disequilibria were found in the two sub-populations. Microsatellite diversity at both single- and two-locus levels is affected by microclimatic environment. The evidence reflects effects of ecological stresses and natural selection on SSR diversity, resulting presumably in adaptive structures.
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Introduction

The previous studies of ecological-genetic interface using allozyme (protein-coding region) (Nevo *et al*, 1982, 1988; Nevo and Beiles, 1989) and randomly amplified polymorphic DNAs (Li *et al*, 1999) suggested that ecological stresses might direct genetic diversity at macro- and microgeographic scales in *Triticum dicoccoides* (reviewed in Nevo, 1988; 1998; Nevo and Beiles, 1989). However, in contrast to the nuclear coding allozymes, microsatellites (SSRs) are primarily *non-coding*, regarded by many as neutral markers. Therefore, their ecological and genetic macro- and microgeographic correlates need critical testing to establish their nature and relevance. Here we show effects of microclimatic stresses on adaptive dinucleotide SSR divergence in natural subpopulations of wild emmer wheat at a microscale.

Materials and methods

Plant materials

Triticum dicoccoides is the tetraploid and predominantly self-pollinated progenitor of cultivated wheats (Zohary, 1970). Its genome constitution is AABB, with 28 chromosomes. Annual wild emmer grows over a wide range of altitudes and ecological amplitudes in several steppe-like herbaceous formations in the Tabor oak, *Quercus ithaburensis*, or in the *Q. brantii* open park forest belts (Zohary, 1973) across the Fertile Crescent (Harlan and Zohary, 1966). The Yehudiyya site is located in an open oak park forest of *Q. ithaburensis* at the lower western foothills of the Golan Heights, northeast of the Sea of Galilee, Israel. Sampling *T. dicoccoides* was conducted in 1985 in an area smaller than 1000 m^2 and involved 12 repeated sampling plots (trees and their immediate circumference). Trees were mosaically distributed and separated on average 10 m from each other, and each experimental tree involved two microclimatic niches separated by only a few meters: (1) shade, mostly shaded during daytime under the canopies of oak trees (trees 10–20 m in height, with canopy diameters up to 20 m); and (2) sun, exposed in daytime to continuous sun radiation and drying in the circumference around each tree and between trees (Nevo *et al*, 1988). The soil temperature in the sun niche was almost 10°C higher than in the shade niche. The relative humidity in soil was similar between the shade and sun niches. During the growing season (Oct–May) of *T. dicoccoides* the shade niche is under stresses of lower temperature and lower intensity of radiation in contrast to the sun niche. The two microniches vary significantly in plant formations (Nevo *et al*, 1988). Wild emmer is sparse in the shade under the oak canopy and abundant in the sun. In each pair of niches for each tree, several plants (4–5) were sampled from the shade under the tree canopy, and nearby from the sun niche immediately surrounding the tree circumference. Thus, the 12 trees sampled represent 12 repeated plots, each involving sun-

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shade paired comparison, separated by 2 to 4 meters and extracted from bush and extensive stands of wild emmer involving thousands of individual plants. In total, we examined SSR diversity of 105 individual plants (50 from the shade and 55 from the sun microniches, 4–5 individuals on the average in the shade and 4–5 plants from the sun around each tree).

Analysis of SSR diversity

Genomic DNA was extracted from seedlings using the method of Junghans and Metzlaff (1990). Plaschke *et al* (1995) and Röder *et al* (1995, 1998) described the SSR primers used in this study. Twenty-eight dinucleotide SSR DNA markers (one for each chromosomal arm, see the accompanying paper Li *et al*, 2002) were chosen for the analysis. The procedure used to detect SSR polymorphism followed Plaschke *et al* (1995) and Fahima *et al* (1998). Fragment sizes were calculated using a Fragment 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 fragment sizes and number of repeat units at the corresponding locus in Chinese Spring (see the accompanying paper Li *et al*, 2002).

Data analysis

An estimate of gene diversity was calculated for each locus and sub-population according to Nei (1973). The χ^2-test was used to test significance of differences in allele frequency and linkage disequilibrium. Niche-*specific* alleles are defined as those that significantly predominate in one of the two niches (either shade or sun). Niche-*unique* alleles are defined as those that are present in only one (either shade or sun) of the two niches. The sign test for each pair of shade and sun niches over all 12 trees was used to analyze the differences in repeat number between the two sub-populations; the Hartley *F*-ratio tested the significance of differences between the two sub-populations for the variance in repeat number at each locus. The POPGENE (Yeh *et al*, 1997) and STATISTICA (Statsoft, 1996) programs were used to perform the statistical analyses. A permutation test was also conducted (by 5000 times of random shuffling of the samples) to estimate the significance of niche-specificity of alleles and linkage disequilibria. Deviation or skewness from symmetry (skewness = 0) was tested using *t*-test.

Genetic distance between the shade and sun sub-populations was estimated according to Nei (1972). The original sample set was randomly shuffled 1000 times, maintaining sample sizes for each niche. The genetic distances were also estimated according to Nei (1972) for the 1000 randomly shuffled sample sets.

Results

Twenty-seven of the tested 28 SSR DNA markers (96.4%) produced fragments; locus GWM637 did not amplify any product in all individuals. The GWM332 produced two fragments in all individuals, which were considered two loci named GWM332a and GWM332b. The two loci were completely linked and mapped at the same position in the long arm of chromosome 7A in the mapping population derived from '*T. dicoccoides* × *T. durum*' (Peng *et al*, 2000). Each of the remaining 26 primer pairs produced one fragment in each individual regarded as an allele of one locus. Out of the total 28 loci, two loci (GWM601 and GWM415) were monomorphic in both microclimatic niches, and the remaining 26 loci (92.9%) were polymorphic.

The distribution of alleles at SSR loci

Alleles were defined by number of repeats. Table 1 presents the summary of statistical analyses for allele distribution at 28 SSR loci. One to 13 alleles were amplified per locus, but the number of alleles (NA) per locus was different at the 28 SSR loci between the two niches. The total NA was 172 and 179 in the shade and sun sub-populations, respectively. The shared NA was 159 in the two sub-populations, and allele distributions were different between the two sub-populations. The χ^2-test was used to test for deviation of each allele distribution between the two sub-populations from the null hypothesis H_o: $P_{sun(i)} = P_{shade(i)}$. After excluding rare alleles (defined as 'observed in $\leq$7 individuals' in the entire sample of 105 plants), 20 and six niche-specific alleles were found at $P < 0.05$ and $P < 0.01$ levels, respectively; more shade-specific ($n = 18$) than the sun-specific alleles ($n = 8$) were observed (Table 2). Among these niche-specific alleles, three shade-unique alleles were found: Two shade-unique alleles at loci GWM218 and GWM459 were found under trees no. 1 and 2. Another shade-unique allele at locus GWM368 was found under trees no. 6 and 7. Two sun-unique alleles were also found at loci GWM136 under trees no. 1, 2 and 3, and GWM251 under trees no. 11 and 12. Genotypes within each pair of niches (microniche and tree) were randomly shuffled maintaining the initial sample sizes of each niche. A permutation test based on 5000 randomly shuffled sets revealed that the significance levels of shade and sun specificity were high ($P < 0.005$, 0.003, respectively, Table 2). This result suggests with high confidence the non-randomness of the revealed sub-population differentiation. Moreover, niche-unique alleles under neighbouring trees reflect migration among close trees, but, without strong microclimatic selection, such niche-uniqueness of some alleles cannot be maintained in a mosaic manner under the tree canopy and separated by sun niche between trees.

Gene diversity, H_e (Nei, 1973), based on allele frequencies, also showed obvious differences between the two climatic divergent sub-populations at some loci. The differences in H_e were as high as 0.10–0.19 at some loci, such as GWM99, GWM219, GWM251, GWM332a, GWM361, GWM408, and GWM429 (Table 1). These results indicated that allele distributions appeared to be nonrandom in the two subpopulations separated by only 2–4 meters but associated with sun *vs* shade climatic microniches.

To test how much the distributions of repeat number (allele size) deviate from symmetry in the two microclimatic niches, skewness was calculated for each locus (Table 1). Allele size distributions significantly deviated from symmetry (skewness = zero) at 12 loci ($P < 0.05$–0.00001). In the shade and sun sub-populations, allele distributions were significantly skewed to the right (skewness > 0) at seven and nine loci, and to the left (skewness < 0) at one and two loci, respectively ($P < 0.05$–0.00001, Table 1). Such patterns indicated that frequencies of alleles with relatively short repeats were significantly higher than those with long repeats. Such distribution

Table 1 Statistics of allele distributions at 27 microsatellite loci in the shade and sun sub-populations of *Triticum dicoccoidies* at Yehudiyya

Locus	*Number of alleles*[a]			*ARN*		*Sign test z (n)*[b]	*Variance*		$F_{(df1,df2)}$	*Skewness*		H_e	
	Shade	*Sun*	*Total*	*Shade*	*Sun*		*Shade*	*Sun*		*Shade*	*Sun*	*Shade*	*Sun*
GWM18	0	1	5	37.3	36.9	1.33 (9)	1.69	2.74	1.62*	–0.14	0.60	0.665	0.737
GWM60	0	0	5	25.2	23.9	2.27* (7)	22.22	20.16	1.10	2.41****	2.27****	0.618	0.630
GWM95	0	1	6	16.9	18.4	–0.00 (11)	2.87	2.07	1.39	–0.07	–0.16	0.748	0.782
GWM99	0	0	5	10.6	11.3	0.00 (5)	22.07	43.19	1.96*	2.40****	1.17***	0.578	0.679
GWM120	0	0	6	33.4	33.2	0.89 (5)	2.12	2.52	1.19	0.93	0.41	0.664	0.732
GWM124	1	2	9	53.4	53.1	1.21 (11)	19.41	34.19	1.76*	0.32	0.37	0.812	0.843
GWM136	3	2	8	68.6	71.1	–	72.15	70.55	1.02	–0.11	–0.44	0.798	0.748
GWM162	0	2	4	23.0	21.5	2.04* (6)	3.80	1.75	2.17**	0.57	2.18****	0.465	0.409
GWM169	1	1	9	20.4	20.1	1.23 (6)	10.81	10.24	1.06	1.24**	1.34****	0.806	0.793
GWM186	0	0	6	23.0	24.2	1.79† (5)	12.75	11.62	1.09	0.55	–0.62	0.664	0.726
GWM218	1	1	9	24.2	23.7	0.35 (8)	20.10	20.52	1.02	–0.21	–0.22	0.818	0.779
GWM219	1	0	8	31.0	23.6	2.67** (9)	83.81	24.48	3.42***	0.53	2.28****	0.799	0.667
GWM251	0	1	6	26.1	25.8	0.76 (7)	2.94	3.54	1.20	0.96	1.19	0.595	0.727
GWM294	1	1	13	24.4	23.2	0.60 (11)	9.56	18.45	1.93*	0.07	0.17	0.862	0.887
GWM332a	0	0	3	18.4	18.2	–0.00 (5)	0.27	0.55	2.03**	0.29	–0.42	0.441	0.632
GWM332b	1	3	13	39.9	39.8	–0.32 (10)	127.01	97.62	1.30	0.60	0.02	0.858	0.889
GWM340	0	0	6	33.8	32.7	2.67** (9)	11.41	8.22	1.39	–1.42***	–1.44	0.819	0.806
GWM361	1	0	5	23.0	22.0	2.04* (6)	2.78	0.56	4.96***	1.89***	1.45***	0.550	0.393
GWM368	2	0	11	31.7	23.9	2.67** (9)	65.52	41.23	1.59	–0.01	1.37*	0.876	0.804
GWM389	0	0	6	33.5	32.8	1.23 (6)	5.50	5.07	1.08	–2.60	–3.10*	0.763	0.698
GWM408	0	1	7	27.1	27.9	–0.35 (8)	5.91	10.18	1.72*	1.29***	0.08	0.692	0.793
GWM415	0	0	1	19.0	19.0	–	0	0	–	–	–	0	0
GWM429	0	1	5	13.2	12.8	–0.00 (3)	3.12	2.74	1.14	3.89***	4.77****	0.529	0.416
GWM459	1	1	11	25.4	25.1	0.32 (10)	23.77	21.57	1.10	0.65	0.23	0.861	0.837
GWM537	0	0	6	37.5	37.4	1.51 (7)	33.04	8.12	4.07***	–1.38**	–2.31***	0.690	0.750
GWM540	0	1	8	18.4	19.0	0.41 (6)	4.74	5.15	1.09	1.50***	1.07*	0.730	0.782
GWM577	0	1	10	33.1	33.9	1.77† (8)	55.48	62.29	1.12	0.68	–0.04	0.772	0.853
GWM601	0	0	1	17.0	17.0	–	0	0	–	–	–	0	0
Overall	13	20	192									0.655	0.688

[a]Shade = shade alleles are those observed only in the shade samples; sun = sun alleles are those observed only in the sun samples.
[b]Sign test was performed for the pairs of ARN in the shade and sun niche across the 12 trees; *n* = number of non-ties.
The ARN difference was not tested for locus GWM136 due to missing data.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.00001$, †$P < 0.10$.

Table 2 Summary of permutation test for niche-specificity of alleles (excluding rare alleles observed in ≤7 individuals) at microsatellite loci of *Triticum dicoccoides* in the sunny and shady microclimatic niches in Yehudiyya, Israel

Number of niche-specific alleles				*Significance of niche-specificity*[b]
Significance level (χ^2-test)[a]	*Shade*	*Sun*	*Total*	
0.01	5	1	6	<0.005
0.05	13	7	20	<0.003
Total	18	8	26	

[a]Test for homogeneity of allele frequencies between the shady and sunny niches.
[b]Significance was obtained in permutation test of 5000 random shuffling sample sets.

appears not to meet the null hypothesis of pure random drift. Out of 100 cases, the skewness may be significant by chance in 5% of cases. According to the binomial distribution, out of our 54 (2×27, two-tailed test) cases, the probability of obtaining significance in 12 cases by chance was very low (0.00006). Opposite direction of skewness was also observed at three to four loci between the shade and sun sub-populations (Table 1).

Sign test over each pair of shade and sun niches around all 12 trees showed that average repeat numbers (ARN) were significantly larger at nine loci in the shade ($P < 0.05$–0.01, Table 1). Significantly, larger variances in repeat number were found in the sun than in the shade sub-population at six loci (GWM18, GWM99, GWM294, GWM124, GWM332a, and GWM408), and an opposite pattern was observed at four other loci (GWM162, GWM219, GWM361, GWM537). These results also indicated significant differences in allele (repeat number) distributions between the two subpopulations.

Genetic distance

The estimate of genetic distance (Nei, 1972) was 0.1057 between the shade and sun subpopulations. To test the significance of the genetic divergence represented by this estimate, the original sample set was randomly shuffled 1000 times maintaining sample size for each tree and niche. Genetic distance between the two subpopulations was calculated for each randomized set. Among the 1000 sets, no case showed larger genetic distance than the observed value, and most of the cases showed about half of the observed genetic distance. Thus, the significance of the observed genetic distance (0.1057) was at least $P < 0.001$. This permutation test indicates that genetic divergence at SSR loci is substantial between the neigh-

bouring shade and sun subpopulations, only a few meters apart.

Discriminant analysis

Forward stepwise discriminant analysis was performed to distinguish individuals from the two microclimatic niches according to multilocus analysis including the 25 polymorphic loci (excluding GWM136 because of missing data). The result indicated that five (GWM18, GWM251, GWM332a, GWM368, GWM459) out of 25 SSR loci were sufficient to correctly classify 82% of 105 individuals into their original microclimatic sun and shade niches. The difference between the distribution center of the two subpopulations was highly significant ($F_{(5,99)} = 15.30$, $P < 0.00005$). Even with only two (GWM169, GWM332a) of the 25 loci, 70% of 105 individuals could be correctly assigned to their original microniches. Here too, the difference between the centroids of the two subpopulations was also highly significant ($F_{(2,102)} = 14.28$, $P < 0.00005$). These results clearly suggest that the observed genetic differentiation at SSR loci is substantial, niche-associated, and therefore, ecologically determined.

Linkage disequilibria (LD) between SSR loci

Triticum dicoccoides is predominantly a self-pollinating plant (about 99%), hence its effective population size is expected to be smaller than those of outcrossing plants and animals. Even without any selection, a small effective size would result in linkage disequilibria, LD (Ohta and Kimura, 1969). However, this is a minor effect. The major effect is that in an outcrosser LDs are reduced in each generation by crossing over between linked loci. The effect of recombination is very low in an inbreeder. It is of interest from the viewpoint of population genetics to investigate how LDs exist between SSR loci in this largely (but not completely) selfing species across the two microclimatic niches. In the present study, 16847, 13530, and 14541 possible pairwise associations were found in the total population, the shade and sun subpopulations, respectively. Of these, 18.0, 21.6, and 14.2 percent were significant (χ^2-test, $P < 0.05$) LDs in the total population, shade and sun subpopulations, respectively. Because rare alleles may cause biased estimation of LD (Lewontin, 1995), further analyses were performed excluding the rare alleles (observed in $\leq$7 individuals in the whole population). In the shade niche, 86%, 90%, and 94% of 1089, 594, and 267 LDs at $P < 0.05$, 0.01, and 0.001 significant levels were niche-*specific* (Table 3). Permutation test, based on 5000 randomly shuffled sets of the initial data, showed high significance of the LD shade-specificity ($P < 0.003$–0.009, Table 3). In the sun subpopulation, after excluding rare alleles, 608, 296, and 131 LDs were also found to be significant at the levels of 0.05, 0.01, and 0.001, respectively. Among these significant LDs, 74–88% were sun-*specific*. However, the permutation test did not show significance of this LD sun-specificity (Table 3). These results suggest that the possibility of observing shade-specific LDs by chance was very low, the shade-specific environment may be responsible for the LD interniche specificity.

The log-linear model test showed that niche effect was very significant ($\chi^2_{df=1} = 34.37$, $P < 0.000005$), whereas, genome effect was negligible ($\chi^2_{df=1} = 0.25$, $P > 0.50$). Linkage itself between loci within chromosomes also seemed not to affect percentages of significant LDs in both subpopulations, reflecting the fact of the higher importance of selfing as compared to linkage.

Discussion

Microclimatic allelic divergence at SSRs in the shade and sun

This study demonstrated that interniche allele distributions at several SSR loci were significantly associated with microclimatic niches of *T. dicoccoides*. Microsatellite diversity in ARN, variance in repeat number (σ^2) and numbers of alleles per locus (A), were significantly affected by microclimatic niche. Excluding rare alleles, niche-*specific* and niche-*unique* alleles were still identified in both sub-populations. In particular, more niche-*specific* and niche-*unique* alleles were observed in the shade subpopulation. The observed niche-*unique* alleles clustered in neighbouring trees. This pattern most probably reflects migration. In such a small area, as suggested by Golenberg (1986) and Li *et al* (1999), seed dispersal by ants, rodents and human activities cannot be ignored. However, if there were not strong microclimatic selection in such a small area of only a few meters between the sun and the shade, the observed SSR differentiation might not have been maintained. Indeed, one would expect that without microclimatic selection, these niche-*unique* alleles should be spread over the neighbouring areas, because a small amount of migration or gene flow is sufficient to swamp the differentiation that arises from the random drift of non-selective genes (Lewontin, 1974). Therefore, we can assume that the revealed patterns may reflect an adaptation to microclimatic specificity of the two very close microclimatic niches. The lower temperature and intensity of radiation in the shade niche, and low moisture stress in the sun niche, may select *specific* or *unique* alleles of the subpopulations of *T. dicoccoides* as earlier demonstrated for nuclear allozyme alleles (Nevo *et al*, 1988). The importance of the present study is the demonstration of possible selection on the *non-coding* genomic regions.

Microclimatic differentiation of SSR linkage disequilibria in the shade and sun

LD can arise from natural selection, or by chance from population sub-division, hitchhiking (selfing and/or linkage), or founder effects. This study showed that the majority of the observed significant LDs were niche-*specific*. Permutation test demonstrated that the niche-specificity was highly significant in the shade, but not in the sun, subpopulations. These results suggest that the shade-specific linkage disequilibria do not arise from stochastic events, but from shade unique microclimate presumably owing to lower temperature and solar radiation (Table 1 in Nevo *et al*, 1988).

Niche-*specificity* was found at single- and two-locus levels in the *shade* subpopulation, suggesting that natural selection may act upon SSR variation, exactly as it does on allozymic diversity where we earlier demonstrated overall (sun + shade), sun and shade linkage disequilibria, suggesting niche-specific selection (Nevo *et al*, 1988). Nevertheless, even the small amount of outcrossing in wild cereals, especially in humid regions (Brown *et al*, 1978), can provide raw material for recombination and clonal selection, particularly in a widespread population

Table 3 Permutation test for niche-specificity[a] of linkage disequilibria (LD) at microsatellite loci of *Triticum dicoccoides* in the sunny and shady niches in the Yehudiyya, Israel

Niche	*Significance level (χ^2-test)*	*Total LDs*	*Proportion of niche-specific LDs*		*No. cases $P_e \geq P_o$*	*Significance of niche-specificity*
			In samples (P_o)	*In 5000 permutations (P_e)*		
Without rare alleles ($n \leq 7$)						
Shade	0.001	267	0.94	0.52–0.96	45	<0.009
	0.01	594	0.90	0.49–0.93	22	<0.005
	0.05	1089	0.86	0.55–0.87	13	<0.003
Sun	0.001	131	0.88	0.68–0.98	2352	NS
	0.01	296	0.79	0.60–0.95	3312	NS
	0.05	608	0.74	0.57–0.90	3502	NS

[a]Permutation test was performed by 5000 random shuffling samples;
[b]Alleles observed in ≤7 individuals in the whole population were regarded as rare alleles.
NS = not significant.

such as that of Yehudiyya involving thousands of wild emmer individual plants organized in a sun/shade mosaic pattern.

It is important to avoid the pitfall of neighbouring effect in the attempt to separate random (founder effect) from non-random (niche structure) factors. Our present data at least partly overcome this problem, since it contains a series of repetitive plots, each with an oak tree canopy (shade) and its surrounding intertree space (sun). The mosaic oak tree structure of our experimental design involving 12 repetitions safeguard, at least partly, from genetic neighborhood effects. The genetic divergence of sun *vs* shade found here is, therefore, unlikely to be the result of stochastic processes. Edaphic and topographic microgeographic SSR divergences were also found in our other two populations of *T. dicoccoides* from two microsites, Tabigha (Li *et al*, 2000a) and Ammiad (Li *et al*, 2000b).

SSR variation and adaptation

The observed microclimatic SSR diversity may imply an adaptation to the shade or sun niche. There is evidence that SSR sequences can serve functional roles as regulatory elements (Karlin *et al*, 1998; Kashi and Soller 1999; King and Soller, 1999) and this is discussed in the accompanying paper (Li *et al*, 2002).

Viewed as functional elements of the genome, the special characteristics of SSRs as mutational hotspots have led to the proposal that SSRs may be a major source of genetic diversity and evolutionary adaptation (Kashi *et al*, 1997; Kashi and Soller, 1999; Trifonov, 2000). Therefore, the niche-unique alleles or linkage disequilibria may result from an adaptation of wild emmer plant to the unique sun or shade niche.

If so, this study complements the one on allozyme diversity (Nevo *et al*, 1988) in demonstrating that both genomic *coding* and *non-coding* regions are subjected to natural selection. This hypothesis is testable by transplant experiment between the sun and shade genotypes and by genetic mapping.

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References

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

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

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

Harlan JR, Zohary D (1966). Distribution of wild emmer wheat and barley. *Science* **153**: 1047–1080.

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

Karlin S, Campbell AM, Mrazek 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 in Genet* **13**: 74–78.

Kashi Y, Soller M (1999). Functional roles of microsatellites and minisatellites. In: Goldstein DB, Schlotterer C (eds) *Microsatellites: Evolution and Application*, Oxford University Press: Oxford. pp 10–23.

King AG, 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 perspective, papers in honor of Eviatar Nevo*, Kluwer Academic Publishers: The Netherlands. pp 65–85.

Lewontin RC (1974). *The Genetic Basis of Evolutionary Change*. Columbia University Press: New York.

Lewontin RC (1995). The detection of linkage disequilibrium in molecular sequence data. *Genetics*, **140**: 377–388.

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

Li YC, Fahima T, Peng JH, Röder MS, Kirzhner VM, Beiles A *et al* (2000a). Edaphic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides,* at a microsite: Tabigha, Israel. *Theor Appl Genet* **101**: 1029–1038.

Li YC, Röder MS, Fahima T, Kirzhner VM, Beiles A, Korol AB, Nevo E (2000b). Natural selection causing microsatellite divergence in wild emmer wheat at the ecologically variable microsite at Ammiad, Israel. *Theor Appl Genet* **100**: 985–999.

Li YC, Fahima T, Röder MS, Beiles A, Korol AB, Nevo E (2002). Genetic effects on microsatellite diversity in wild emmer wheat (*Triticum dicoccoides*) at the Yehudiyya microsite, Israel. *Heredity* (in press)

Nei M (1972). Genetic distance between populations. *Am Nat* **106**: 283–292.

Nei M (1973). Analysis of gene diversity in subdivided population. *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, Beiles A, Krugman T (1988). Natural selection of allozyme polymorphisms: a microgeographic climate differentiation in wild emmer wheat (*T. dicoccoides*). *Theor Appl Genet* **75**: 529–538.

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

Ohta T, Kimura M (1969). Linkage disequilibrium due to random genetic drift. *Genet Res* **13**: 47–55.

Peng HJ, Korol AB, Fahima T, Roder MS, Ronin YI, Li YC *et al* (2000). Genome-wide molecular genetic map, quasi-linkage and negative interference in wild emmer wheat, *Triticum dicoccoides*. *Genome Res* **10**: 1509–1531.

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.

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

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

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

Trifonov EN (2002). Tuning function of tandemly repeating sequences: a molecular device for fast adaptation. *Gene* (in press).

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.