SITE INFLUENCES ON THE GENETIC VARIATION AND STRUCTURE OF *PINUS HALEPENSIS* MILL. PROVENANCES[1]

Leonid Korol[2], Galina Shklar[2] & Gabriel Schiller [2, 3]

[1)] Contribution Number 138/2000 of the Agricultural Research Organization, The Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel.

[2)] Department of Agronomy and Natural Resources, Forestry Section, Agricultural Research Organization, The Volcani Center, P. O. Box 6, Bet Dagan, 50250 Israel.

[3)] To whom correspondence should be sent: E-mail: vcgabi@volcani.agri.gov.il.

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ABSTRACT

We tested whether the F_1-offsprings rose from seeds collected in natural native Israeli and overseas populations of *Pinus halepensis* Mill. planted under semi-arid environmental conditions exhibit genetic diversity and structure similar to those of their parental origin populations. Allele frequencies and genotypes of trees that had survived since planting 15 years ago, and those in the natural populations of origin were determined by means of starch gel electrophoresis technique. Use was made of the haploid megagametophyte tissue of the pine seeds. The percentage of polymorphic loci increased from an average of 40 % in the natural populations to 46 % in the progenies, and the average observed heterozygosity rose from 0.118 to 0.167. The largest changes in diversity, heterozygosity and fixation index occurred in the population that had originated in the highest and wettest environment, whereas only minor changes occurred in a population that had originated in a relatively drier environment.

Dry-land afforestations in places outside the natural distribution area of a species can be looked upon as peripheral populations with their higher genetic diversity conferred by selection, and thus having more resistance to extreme conditions. Therefore, such afforestations should be treated as a biogenetic resource available for rehabilitation and restoration of damaged Aleppo pine ecosystems.

Keywords: Aleppo pine, population genetics, selection stress influence, genetic diversity.

INTRODUCTION

Pinus halepensis Mill. (Aleppo pine) is widely distributed around the Mediterranean and grows in regions with differing climates (EMBERGER *et al.* 1963; PANETSOS 1981). This species is among the most drought-tolerant of Mediterranean pine species (OPPENHEIMER 1967) and is adapted to dry Mediterranean climates (NAHAL 1981), therefore, it is used worldwide in reforestation and afforestation of degraded areas under semi-arid and arid climates.

As a result of the FAO-IUFRO project on Mediterranean conifer species genetic resources (MORANDINI 1976), Aleppo pine seed material from various geographic regions within its circum-Mediterranean distribution became available, and thus facilitated two different genetic approaches. (i) The establishment of provenance (progeny) trials in many countries to determine and compare the success (survival, growth) of the various provenances (ecotypes, genotypes) in a variety of environments. (ii) Analysis of circum-Mediterranean and local geographic, morphological and genetic diversity of Aleppo pine populations (SCHILLER 2000). Results of earlier research on seed and seedling characters of native Israeli populations (e.g., MELZACK *et al.* 1981 1982) suggested a south-north cline of 100-seed weight and number of cotyledons. Furthermore, data of direct count (observed) and/or Hardy-Weinberg's expected heterozygosity of native Israeli populations (e.g., GRUNWALD *et al.* 1986) have shown a south-north cline in heterozygosity (SCHILLER & WAISEL 1989), which according to NEVO (1983) also means an inverse relationship to the annual rainfall in this geographic region.

In Israel, one of the provenance trials was planted within the Yatir afforestation project in the southern foothills of the Judaean Hills, bordering the northern part of the Israeli Negev desert (31° 21' N, 35° 02' E, 630 m a. s. l.). This region is characterized by higher solar radiation, lower relative air humidity and lower levels of precipitation of only 278 ± 89 mm, 35-year-average annual rainfall, in comparison with more than 500 mm in regions in which natural Aleppo pine forests grow in Israel. We assume that climatic natural selec-

tion is one of major evolutionary driving forces shaping population's genotypic structure. 12 years after planting average survival rates of the provenances planted was 73 %; survival rate of the Bet J'ann population was 53 % only. 15 years after planting, which lately included two years of very sever drought of only 150 mm annual rainfall, average survival rate declined to 44 % and 27 %, respectively. The existence, at the edge of the desert, of a provenance trial planted in 1985 with 20 F_1-offspring populations enabled us to quantify short-term changes, at the allozyme level, among populations within provenances in response to environmental conditions. Therefore, the aim of this study was to investigate possible changes of isoenzyme variations in three Aleppo pine F_1-offspring populations derived from parent natural forest populations from differing geographic regions and site conditions in Israel and Greece.

This study was carried out within the framework of the European Community International Cooperation with Developing Countries (INCO-DC) research project on "Global, Physiological and Molecular Responses to Climatic Stresses of Three Mediterranean Conifers".

MATERIALS AND METHODS

Seed Materials

The genetic diversity and structure were analyzed by means of isoenzyme starch gel electrophoresis applied to the megagametophyte tissue of seeds, i.e., a haploid maternal tissue (CONKLE *et al.* 1982). Table 1 displays data on the seed origins:

1. Two natural native Israeli Aleppo pine forests –Mt. Carmel and Bet J'ann. Seed material was collected in these forests in 1980. Seed material of each tree was kept separately. Part of this seed material was used for genetic analysis, and a part was bulked for the production of seedlings for the Yatir forest provenance trial planted in 1985. In 1995 recollection of seeds, as much as possible from the same trees as in 1980, separated by several tens of meters and randomly selected and marked, was done; five cones per tree were collected. We assume that these samples correctly represent the populations in their allele frequencies and structure. The seeds extracted were kept separately for each tree.

2. Istiaia provenance (Greece). A Bulked seed lot resulting from the IUFRO-FAO seed collections in 1976 (MORANDINI 1976) from an unknown number of trees and mod of collection. Part of this seed lot was used to raise F_1-offsprings that were planted in the Yatir forest provenance trial in 1985; the other part of the seed lot was kept.

3. Cones collected in 1997 in three first-generation progeny (F_1 -offsprings) populations, mentioned above planted in 1985 in the Yatir forest provenance trial. Cones were collected from randomly selected 27 trees per population in all the 18 replications of the provenance trial; the seeds extracted were kept separately for each tree. All seeds were kept at –5 °C in polyethylene bags.

Electrophoresis

Seed preparation and germination, enzyme extraction and horizontal starch gel electrophoresis were performed according to CONKLE *et al.* (1982). Analyses were performed with eight haploid megagametophytes per mother tree (the probability of detecting a heterozygous tree was 0.992). Four different electrophoresis buffer systems were used to analyze 14 enzyme systems

Table 1. Provenances and populations of *Pinus halepensis* analyzed

No	Seed source name	Abbreviation	Longitude E	Latitude N	Altitude (m a.s.l.)	Mean annual rainfall (mm)	No. of analyzed trees	% Survival after 15 years
	Native population							
1	Istiaia	Ist. N	23° 18'	38° 58'	15–250	-	76	
2	Beit J'ann (Mt. Miron)	BJ. N	35° 23'	32° 58'	850	925	27	
3	Carmel (Mt. Carmel)	Car. N	35° 02'	32° 43'	450	750	66	
	Planted at Yatir							
4	Istiana	Ist. Y	35° 02'	31° 21'	630	261	26	42.7
5	Beit J'ann	BJ. Y	35° 02'	31° 21'	630	261	16	27.0
6	Carmel	Car. Y	35° 02'	31° 21'	630	261	18	42.5

Table 2. Enzyme systems analyzed, E. C. numbers and buffer systems used

Enzymes	Abbreviation	Locus	E. C. No	Buffer System
Alanine aminopeptidase	AAP	1	3.4.11.2	I
Aconitase	ACO	1	4.2.1.3	III
Acid phosphotase	ACP	1	3.1.3.2	IV
Alcohol dehydrogenase	ADH	1,2	1.1.1.1	I
Catalase	CAT	2	1.11.1.6	II
Glutamate dehydrogenase	GDH	1	1.4.1.3	II
Glutamate-oxaloacetate transaminase	GOT	1,2,3	2.6.1.1	II
Isocitric dehydrogenase	IDH	1	1.1.1.42	III
Leucine aminopeptidase	LAP	1	3.4.11.1	I
Malate dehydrogenase	MDH	1,2,3,4	1.1.1.37	IV
Menadione reductase	MNR	1,2	1.6.99.2	I
Phosphoglucose isomerase	PGI	1,2	5.3.1.9	IV
Phosphoglucomutase	PGM	1,2	2.7.5.1	I
6-Phosphogluconate dehydrogenase	6PGD	2,3	1.1.1.44	II

extracted from tissue that was homogenized in a grinding plate (KOROL & SCHILLER 1996) (Table 2). After electrophoresis, the gels were sliced and stained according to CONKLE *et al.* (1982).

Data analysis

By applying the BIOSYS–1 computer program, version 1.7 (e.g., SWOFFORD & SELANDER 1981) calculations of intra- and inter-population genetic diversity were performed. Data of the allele frequencies were used to calculate the proportions of total diversity resulting from differences between populations (G_{ST} %) (NEI 1973, 1978). G_{ST} values were also calculated for each polymorphic locus and then averaged over all loci in the populations and in groups (HAMRICK & GODT 1989). The value of population genetic differentiation F_{IS} was calculated as the weighted average of $F_{IS,O}$ for all alleles and all loci in each population according to WRIGHT (1965) and NEI (1977). Genetic distances between populations were computed by means of NEI's (1978) formula and the chord distance by means of CAVALLI-SFORZA & EDWARDS (1967) formula. G_h index (i.e., number of heterozygous genotypes per tree) was calculated by division of the number of heterozygous genotype by the number of trees.

An ANOVA algorithm and SigmaStat statistical software (SPSS Inc.) were used for multiple comparisons and testing the significance of the differences among populations within provenances and groups.

RESULTS

Isoenzyme patterns

Fourteen enzyme systems were examined by starch gel electrophoresis and all were found to be polymorphic at least in one locus. Allele frequencies are presented in Table 3; they were numbered as in previous studies (KOROL & SCHILLER 1996).

Within-population genetic variation.

Intra-population diversity was estimated by five genetic indices that are presented in Table 4. We assume that the high number of loci analyzed compensates for the small sample size. The mean value of number of alleles per locus for the six populations investigated was 1.68. The change in the mean value of number of alleles per locus from 1.3 in the natural Bet J'ann population to 1.5 in the F_1-offspring population at Yatir forest is statistically significant by means of Student's t-test. In all cases, the average percentage of polymorphic loci (P %) was lower within the natural sites than in the F_1-offspring populations in the Yatir forest; and the largest difference was also found between the two sites of the Bet J'ann provenance: it increased from 12 to 40 %.

Differences between the parental natural populations and their F_1-offsprings planted in the Yatir forest in the observed and gene diversity i.e., expected heterozygosity, H_{obs} and H_{exp}, were found (Table 4). In general, levels of genetic diversity were higher in F_1-offsprings planted in Yatir than in the natural parental populations. In the Bet J'ann provenance, observed and expected heterozygosity increased from 0.049 and 0.054 in the natural population to 0.160 and 0.133, respectively, in the Yatir forest. The G_h index has the tendency to be lower in the natural populations. Thus, genetic inter-site divergence was larger under the climatic stressful conditions.

Deviation from Hardy-Weinberg's equilibrium was tested by means of comparison between the observed

Table 3. Allele frequencies in populations and enzyme system analyzed.

		Natural			Yatir		
Locus	Allele	Istiaia	Bet J'ann	Carmel	Istiaia	Bet J'ann	Carmel
	N	76	27	66	26	16	18
Aap	1	0.572	0.370	0.333	0.596	0.094	0.556
	2	0.059	0.630	0.667	0.308	0.906	0.222
	3	0.343	0.000	0.000	0.096	0.000	0.222
	0	0.026	0.000	0.000	0.000	0.000	0.000
Aco	1	0.618	1.000	0.826	0.654	0.562	0.833
	2	0.382	0.000	0.174	0.327	0.438	0.167
	3	0.000	0.000	0.000	0.019	0.000	0.000
Acp	1	0.947	1.000	1.000	0.788	1.000	0.778
	2	0.053	0.000	0.000	0.212	0.000	0.139
	3	0.000	0.000	0.000	0.000	0.000	0.083
Adh-1	1	0.757	1.000	1.000	0.846	1.000	0.806
	2	0.217	0.000	0.000	0.154	0.000	0.194
	0	0.026	0.000	0.000	0.000	0.000	0.000
Adh-2	1	0.632	0.926	0.871	0.692	0.906	0.944
	2	0.125	0.074	0.091	0.039	0.000	0.056
	3	0.072	0.000	0.000	0.000	0.000	0.000
	0	0.171	0.000	0.038	0.269	0.094	0.000
Cat-2	1	0.993	0.463	0.864	0.962	0.656	1.000
	2	0.007	0.537	0.136	0.038	0.344	0.000
Gdh	1	1.000	1.000	0.992	0.885	0.750	1.000
	2	0.000	0.000	0.008	0.115	0.250	0.000
Got-1	1	1.000	1.000	1.000	0.981	1.000	1.000
	2	0.000	0.000	0.000	0.019	0.000	0.000
Got-2	1	1.000	1.000	0.947	1.000	1.000	1.000
	2	0.000	0.000	0.053	0.000	0.000	0.000
Got-3	1	1.000	1.000	0.939	0.904	1.000	1.000
	2	0.000	0.000	0.053	0.096	0.000	0.000
	3	0.000	0.000	0.008	0.000	0.000	0.000
Idh	1	0.954	1.000	1.000	1.000	0.969	1.000
	2	0.000	0.000	0.000	0.000	0.031	0.000
	3	0.046	0.000	0.000	0.000	0.000	0.000
Lap-1	1	0.309	0.963	0.871	0.635	0.906	0.944
	2	0.664	0.000	0.000	0.346	0.000	0.028
	0	0.027	0.037	0.129	0.019	0.094	0.028
Mdh-1	1	0.993	1.000	1.000	1.000	1.000	1.000
	2	0.007	0.000	0.000	0.000	0.000	0.000
Mdh-2	1	0.000	0.037	0.023	0.000	0.000	0.083
	2	0.987	0.963	0.977	1.000	1.000	0.917
	0	0.013	0.000	0.000	0.000	0.000	0.000

Table 3. Allele frequencies in populations and enzyme system analyzed.

		Natural			Yatir		
Locus	Allele	Istiaia	Bet J'ann	Carmel	Istiaia	Bet J'ann	Carmel
	N	76	27	66	26	16	18
Mdh-3	1	0.921	0.981	0.735	0.788	0.812	0.667
	0	0.079	0.019	0.265	0.212	0.188	0.333
Mdh-4	1	0.283	0.019	0.167	0.346	0.281	0.222
	2	0.717	0.981	0.833	0.654	0.719	0.778
Mnr-1	1	0.053	0.000	0.197	0.385	0.344	0.111
	2	0.940	1.000	0.795	0.615	0.656	0.889
	3	0.007	0.000	0.008	0.000	0.000	0.000
Mnr-2	1	1.000	1.000	1.000	0.981	1.000	0.972
	2	0.000	0.000	0.000	0.019	0.000	0.028
Pgm-1	1	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-2	1	0.322	0.000	0.106	0.865	0.063	0.333
	2	0.678	1.000	0.894	0.135	0.937	0.583
	3	0.000	0.000	0.000	0.000	0.000	0.084
Pgi-1	1	1.000	1.000	1.000	0.962	1.000	1.000
	2	0.000	0.000	0.000	0.038	0.000	0.000
Pgi-2	1	0.803	1.000	0.955	0.942	1.000	0.972
	2	0.151	0.000	0.045	0.058	0.000	0.028
	3	0.046	0.000	0.000	0.000	0.000	0.000
6pgd-1	1	1.000	1.000	1.000	1.000	1.000	1.000
6pgd-2	1	0.842	1.000	1.000	0.846	1.000	1.000
	2	0.158	0.000	0.000	0.154	0.000	0.000
6pgd-3	1	0.809	1.000	0.992	0.846	0.969	0.917
	2	0.191	0.000	0.008	0.154	0.031	0.083

and expected frequencies of genotypes. For the F_1-offspring trees growing in the Yatir forest, the majority of loci were in equilibrium; the deviation from heterozygote appearance is not significant. On the contrary in the parental natural populations, most of the loci deviated significantly ($P<0.05$) from Hardy-Weinberg's equilibrium, which indicates significant heterozygote deficiency. Only in the *Gdh*, *Mdh*–1 and *Mdh*–2 loci, non-significant deviation from equilibrium was revealed with probability values (P) of 0.457, 0.541 and 0.131, respectively. The number of loci indicating hetrozygotes excess within the provenances growing in Yatir increased to nine. A global test over all populations and loci was significant: $\chi^2 = 1422.1$, d.f. =185, $P<0.01$.

Excess or deficiency of heterozygotes for each locus and each population were examined by means of Wright's fixation Index, i.e., "inbreeding coefficient". The results presented in Table 5 show different heterozygote frequencies in each population. However, when Fis values where pooled according to location (Natural versus Yatir), two distinct groups emerged. Within the Yatir group (F_1-offsprings populations 4, 5, 6) most of the F_{IS} values were lower than zero, which expresses their excess of heterozygotes. Within the second group (parental natural populations 1, 2, 3) most F_{IS} values were positive, which reflects deficiency in heterozygotes. Comparison between the mean F_{ISP} of these two groups showed a statistically significant P value of 0.0017. The mean F_{ISP} estimate of the parental natural population was 0.098 and that for the F_1-offspring populations in Yatir –0.090, the difference between the two means reaching 0.188. The differences in the fixation indexes between pairs, i.e., parental

Table 4. Genetic variability of the analyzed populations.

Pop.#	Name of population	No of trees	*A1/L*	$P_{\%}$ *	H_{obs}	H_{exp} **	*Gh*
	Native populations						
1	Istiaia (GR)	76.0	2.0	52.0	0.138	0.188	3.45
2	Mt. Beit J'ann (IS)	27.0	1.3	12.0	0.049	0.054	1.22
3	Mt. Carmel (IS)	66.0	1.7	44.0	0.116	0.120	2.91
		Mean	*1.7*	*36.0*	*0.101*	*0.121*	*2.88*
	Planted at Yatir						
4	Istiaia (GR)	26.0	1.9	60.0	0.220	0.214	5.27
5	Mt. Beit J' ann (IS)	16.0	1.5	40.0	0.160	0.133	4.00
6	Mt. Carmel (IS)	18.0	1.7	48.0	0.144	0.153	4.11
		Mean	*1.7*	*49*	*0.175*	*0.167*	*4.43*

A1/L - mean number of alleles per locus; $P_{\%}$ - percentage of polymorphic loci. * - a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95; H_{obs} - observed heterozygosity; H_{exp} - expected level of heterozygosity. ** unbiased estimate (see NEI 1978).

Table 5. Fixation indices of polymorphic loci in the six analyzed populations of *P. halepensis*.

Locus	Population					
	Natural			Yatir		
	Istiaia	Bet J'ann	Carmel	Istiaia	Bet J'ann	Carmel
Aap	0.141	0.047	0.045	0.218	-0.103	0.438
Aco	0.108	-	-0.000	0.008	0.238	-0.200
Acp	-0.056	-	-	-0.268	-	-0.205
Adh-1	-0.005	-	-	-0.182	-	-0.241
Adh-2	0.857	-0.080	0.214	-0.033	-0.103	-0.059
Cat	-0.007	0.181	-0.158	-0.040	-0.524	-
Gdh	-	-	-0.008	-0.130	-0.333	-
Got-1	-	-	-	-0.020	-	-
Got-2	-	-	-0.056	-	-	-
Got-3	-	-	-0.057	-0.106	-	-
Idh	0.551	-	-	-	-0.032	-
Lap-1	0.516	-0.038	-0.013	-0.048	-0.103	-0.043
Mdh-1	-0.007	-	-	-	-	-
Mdh-2	1.000	-0.038	0.659	-	-	-0.091
Mdh-3	1.000	-0.019	0.495	-0.038	-0.231	0.500
Mdh-4	-0.394	-0.019	-0.200	-0.529	-0.391	-0.286
Mnr-1	-0.056	-	-0.246	0.188	-0.524	-0.125
Mnr-2	-	-	-	-0.020	-	-0.029
Pgm-2	0.789	-	-0.119	0.175	-0.067	-0.026
Pgi-1	-	-	-	-0.040	-	-
Pgi-2	0.165	-	-0.048	-0.061	-	-0.029
Pgd-2	-0.188	-	-	-0.182	-	-
Pgd-3	-0.065	-	-0.008	0.114	-0.032	-0.091
Mean F_{ISP}	0.256	0.005	0.033	-0.052	-0.184	-0.035

Fixation index for a population was computed as weighted average of F_{IS} for all alleles at a locus in each site. $F_{IS} = 1$, only heterozygotes were found., $F_{IS} = 0$, number of observed heterozygotes as expected., $F_{IS} = -$, the locus was monomorphic, F_{ISP} - mean of F_{IS} values for population.

Table 6. Values of genetic diversity (H_s) for each loci, population and group (calculated according to HAMRICK & GODT 1989)

Locus	Natural				Yatir			
	Istaia	Bet J'ann	Carmel	Mean	Istaia	Bet J'ann	Carmel	Mean
Aap	0.5517	0.4662	0.4442	**0.4874**	0.5407	0.1703	0.5923	**0.4344**
Aco	0.4722	0.0000	0.2874	**0.2532**	0.4650	0.4912	0.2782	**0.4115**
Acp	0.1004	0.0000	0.0000	**0.0335**	0.3341	0.0000	0.3685	**0.2342**
Adh-1	0.3792	0.0000	0.0000	**0.1264**	0.2606	0.0000	0.3127	**0.1911**
Adh-2	0.5505	0.1370	0.2316	**0.3064**	0.4473	0.1703	0.1057	**0.2411**
Cat	0.0139	0.4973	0.2350	**0.2487**	0.0731	0.4513	0.0000	**0.1748**
Gdh	0.0000	0.0000	0.0159	**0.0053**	0.2035	0.3750	0.0000	**0.1929**
Got-1	0.0000	0.0000	0.0000	**0.0000**	0.0373	0.0000	0.0000	**0.0124**
Got-2	0.0000	0.0000	0.1004	**0.0335**	0.0000	0.0000	0.0000	**0.0000**
Got-3	0.0000	0.0000	0.1154	**0.0385**	0.1736	0.0000	0.0000	**0.0579**
Idh	0.0878	0.0000	0.0000	**0.0293**	0.0000	0.0601	0.0000	**0.0200**
Lap 1	0.4629	0.0713	0.2247	**0.2530**	0.4767	0.1703	0.1073	**0.2514**
Mdh-1	0.0139	0.0000	0.0000	**0.0046**	0.0000	0.0000	0.0000	**0.0000**
Mdh-2	0.0257	0.0713	0.0449	**0.0473**	0.0000	0.0000	0.1522	**0.0507**
Mdh-3	0.1455	0.0373	0.3895	**0.1908**	0.3341	0.3037	0.4442	**0.3607**
Mdh-4	0.4058	0.0373	0.2782	**0.2404**	0.4526	0.4041	0.3454	**0.4007**
Mnr-1	0.1117	0.0000	0.3291	**0.1469**	0.4736	0.4513	0.1974	**0.3741**
Mnr-2	0.0000	0.0000	0.0000	**0.0000**	0.0373	0.0000	0.0544	**0.0306**
Pgm-1	0.0000	0.0000	0.0000	**0.0000**	0.0000	0.0000	0.0000	**0.0000**
Pgm-2	0.4366	0.0000	0.1895	**0.2087**	0.2335	0.1162	0.5423	**0.2974**
Pgi-1	0.0000	0.0000	0.0000	**0.0000**	0.0731	0.0000	0.0000	**0.0244**
Pgi-2	0.3303	0.0000	0.0860	**0.1387**	0.1093	0.0000	0.0544	**0.0546**
Pgd-2	0.2661	0.0000	0.0000	**0.0887**	0.2606	0.0000	0.0000	**0.0869**
Pgd-3	0.3090	0.0000	0.0159	**0.1083**	0.2606	0.0601	0.1522	**0.1576**
Means	**0.1865**	**0.0527**	**0.1195**	**0.1196**	**0.2099**	**0.1290**	**0.1483**	**0.1624**
s.e.	0.0409	0.0267	0.0285	0.0253	0.0375	0.035	0.0373	0.0300

natural populations versus their F_1-offsprings were statistically significant for two of the three provenances, namely for Istiaia and Bet J'ann with P values of 0.0131 and 0.0199, respectively.

The positive deviation of the F value from zero, i.e., deficits in heterozygotes may have various causes: the Wahlund effect, positive mating among similar genotypes, selection for homozygote genotypes, etc. (e.g., EL-KASSABY *et al.* 1987). In contrast, the F_{IS} indexes of single loci in the F_1-trees growing at Yatir indicate an excess of heterozygotes. Seventeen of the 21 loci showed negative F_{IS} values, and the mean F_{IS} value over all F_1 -populations in this location was negative. Negative F_{IS} values, i.e., excess heterozygotes, may have several causes, of which the preferential one to us is natural selection, and selection during seedlings production, favoring heterozygotes and, consequently increasing the heterozygosity while adaptation.

Differences in the genetic structure

In three of the 14 enzyme systems analyzed, differences in allele frequencies were revealed between the parental natural populations and their F_1-offsprings growing in the Yatir forest provenance trial (Table 3). In these enzyme systems there are loci in which a certain allele is probably more sensitive to stressful conditions. Frequencies of several alleles in the loci *Mdh*–3, *Mdh*–4 and *Pgm*–2 were higher in the F_1-offsprings than in the parental natural populations. In the loci *Mdh*–4 and *Pgm*–2 the difference was related to the alteration of heterozygous genotypes. In the loci *Lap* and *Mdh*–3 changes were related to more frequent homozygous genotypes. Increased frequency of allele2 in the *Aap* locus is an indicator that characterizes the East-Mediterranean group (SCHILLER *et al.* 1986). The mean frequency of this allele among 20 circum-Mediterranean populations was 0.318 (unpublished data); for non-Israeli provenances it was 0.251 and for the Israeli provenances alone, 0.521. Hence, it is

reasonable to assume that the increased frequency of allele 2 in the *Aap* loci was related to adaptation to environmental conditions. Differences between parental natural populations and their F_1 -progenies in the Yatir forest were found in the common allele "1" of the *Pgm-2* locus (Table 3). Similar differences were found in the null allele of the *Mdh–3* locus. The presence in conifers of a null allele in *Mdh* has been reported previously (e.g., GONCHARENKO *et al.* 1993, 1994; SCALTSOYIANNES *et al.* 1994). The frequency of this null allele in the *Mdh–3* locus was always higher in the F_1-trees growing in Yatir than in their parental natural populations. Significant differences existed between sites only in the case of the *Mdh–3* locus.

Linear regression calculated between the F_1-offsprings survival rate (independent variable) and the increase in the percentages of observed heterozygosity, or the number of heterozygous genotypes per tree for each provenance (dependent variables) resulted with negative correlation coefficients of –0.9992 and –0.9978, respectively, with a P value of <0.05. Correlation coefficients of the linear regression between F_1-offspring survival rate and the increase of F_{ISP} values was positive 0.9733, with a P value of <0.05.

Genetic diversity within sites was calculated for each locus, and inter-site genetic divergence was obtained by averaging over all loci and populations (Table 6). Differences in the genetic diversity between the parental natural populations and their F_1-offspring at Yatir forest were significant only in the Bet J'ann provenance ($P = 0.044$). Inter-site genetic diversity was larger in Yatir and the differences between the groups parental natural and Yatir in the mean values of genetic diversity were significant: $P = 0.027$. Genetic diversity values in 15 loci of the 23 (65 %) detected in the provenances growing in the Yatir forest provenance trial had values larger than in the same locus in the parental natural forests. Analysis among loci showed that the differences in the genetic diversity were significant only for the *Gdh* locus. Differentiation between populations was estimated by means of the G_{ST} parameter (analogous to F_{ST}) that showed no significant differences between levels of differentiation. The level of genetic differentiation (G_{ST}) among natural populations was 0.175, and that among the F_1 trees in the Yatir forest was 0.136. Gene variation among populations was about 15 % of the total variation, thus most of the allelic variation (~85 %) resides within individual populations. Low levels of differentiation among populations are usual in conifers (GRUNWALD *et al.* 1986, HAMERICK & GODT 1989).

Genetic distances according to NEI (1978) were very small (0.013–0.080), therefore, we used the chord distances according to CAVALLI-SFORZA & EDWARDS (1967), which are shown in Table 7. The lowest values among the parental natural populations were obtained between the Mt. Carmel and the Bet J'ann populations, which are genetically related (GRUNWALD *et al.* 1986). Values of genetic similarity among these two populations were high, which reflects the genetic similarity in the structures of both populations.

DISCUSSION AND CONCLUSIONS

Random collection of seeds in a panmictic mating system should theoretically produce F_1 progenies identical in their genetic diversity and structure to their parent populations. Although the genetic distance found between provenances (Mt. Carmel, Bet J'ann, Istiaia) within sites (Natural, Yatir) was very small, which indicates their very close genetic relationship, allozyme polymorphism, genetic diversity and heterozygote frequency indicate microsite genetic divergence. Since no known ecological factor except climate could influence the inter-site divergence, changes that occurred in the Yatir provenance trial can, therefore, be explained as presumably being caused by climatic natural selection.

The percentage of polymorphic loci increased from 36 to 49 % (a 36 % increase) and the observed heterozygosity increased from 0.101 to 0.175 (a 73 % increase). The most drastic change in diversity, struc-

Table 7. Genetic diastances among the six populations of *Pinus halapensis* analyzed.

No population	Istaia / Nat.	Bet J'ann/Nat.	Carmel/Nat.	Yatir/Bet J'ann	Yatir/Carmel	Yatir/Istaia
1 Istaia / Natural	-					
2 Bet J'ann/Natural	0.266	-				
3 Carmel/Natural	0.220	0.139	-			
4 Yatir / Bet J'ann	0.251	0.176	0.116	-		
5 Yatir / Carmel	0.174	0.210	0.155	0.204	-	
6 Yatir Istaia	0.159	0.278	0.202	0.217	0.170	-

Genetic distances were measured by means of CAVALLI-SFORZA & EDWARDS (1967) chord distance.

ture and fixation index occurred in the Beit J'ann F_1-population that originated from a very small, isolated stand growing on Mt. Meron under relative favourable site conditions (Table 1). This population established it self naturally later then the survey by CONDER & KITCHENER executed between 1872 and 1877 CONDER & KITCHENER 1880). The population is characterized by a high number of monomorphic loci and very low heterozygosity, which is probably the result of the founder effect, positive mating among similar genotypes and selection for homozygote genotypes. (GRUNWALD *et al.* 1986). Possibly favourable site conditions (low summer temperature, high rainfall, favorable soil and bedrock characteristics) enabled homozygous seedlings to survive and to become mature trees and thus enlarge the population. The last 40 years have seen the establishment of large reforestation projects in Galilee, which subsequently created huge pollen dispersal, whose influence, i.e., addition of alleles, can be found in the F_1 offsprings population in the Yatir forest provenance trial. Furthermore, environmental selection pressure has probably caused the elimination of the homozygous trees from this population and in others too in Yatir; only 27 % of the Bet J'ann F_1-offsprings planted survived, compared with more than 44 % of all the other 20 provenances planted. This elimination would account for the increases in the mean number of alleles per locus, percentages of polymorphic loci, observed and expected heterozygosity (Table 4), and the changes in the fixation index (Table 5).

Levels of genetic variation are presented in Tables 4 and 5. In general the values increased in the populations growing in the provenance trial in Yatir, in other words, genetic diversity is higher under stress full conditions (SAFRIEL *et al.* 1994). We assume that correlation between gene diversity and survival rates with a negative correlation coefficients suggests a model of heterozygosity, when heterozygous individuals being developmentally more stable than their homozygous counterparts (LENER 1954; SOULE 1979). All these indicate that at least some of the inferred changes may be reflected in selective responses to environmental conditions that are also reflected in the low survival rate 15 years after planting. Further natural selection should promote the divergence within provenances at a micro-site (NEVO *et al* 1998). Therefore, theoretically it seems reasonable that micro-site ecological-genetic differences can promote a tendency toward site-specific differentiation for stress-resistance traits. Yatir as a micro-site that causes differentiation is an ecological model for the understanding of long-term evolution. It is obvious that population size, history, and past and present genetic flow values, are important in the determination of genetic heterogenity and structure within and between plant populations. Nevertheless, adaptive selection receives increasing attention: non-selective processes, which cause loss of allele heterozygosity, probably influence all components of population adaptation. The "Evolution Canyon" model enabled the similarity between environmental conditions and plant species diversity to be shown (NEVO 1997). This parallelism between diversity and microhabitats suggests that genetic and physiological diversity represent a complex of adaptive factors related to environmental heterogeneity. Climatic natural selection, imposed through drought stress, appears to be the major differentiating factor. Apparently, the proportion of heterozygotes in the population is related to its adaptive effectiveness, accordingly, stressful conditions may change the adaptive capability of plant populations.

Genetic diversity is the basis of evolutionary processes in nature, and it is apparent within numerous species at the enzyme and DNA levels (NEVO 1998). Adaptation to local environmental conditions has been proposed as an explanation of differences in allele frequencies among populations of forest tree species covering large geographic areas. Genetic differentiation within species has been suggested to relate to microgeographic adaptation (LINHART *et al.* 1981, HAMRICK *et al.* 1989). Correlations between allele frequencies and adaptation to ultramafic soils was established by FURNIER & ADAMS (1986), and correlations between allele frequency and climatic variables such as winter temperature were found by GURIES & LEDIG (1981). Significant correlation coefficients of linear regressions between allele frequencies in several enzyme systems in *Pinus brutia* ecotypes growing in the Taurus Mountains (dependent variable and the prevailing climatic parameters (independent variable) were obtained by KARA *et al.* (1997). Similar significant relations were found among *P. canariensis* populations on the Canary Islands (SCHILLER *et al.* 1999). In the Swiss sub-alpine stands of *Picea abies* and *Fagus sylvatica* populations, relatively large intra-population and inter-population average genetic variations were found, compared with reference populations in Europe (MÜLLER-STARCK 1985).

Another factor whose influence on changes in allele frequency should not be neglected is the "domestication" effect on the plant population. Studied of forest tree populations in their early stage of domestication showed that heterozygosity levels in domesticated populations were similar to or higher than heterozygosity in their natural progenitors (EL-KASSABY & RITLAND 1996). Differences between the natural populations and the first-generation progeny orchards were not significant, but rare localized or private alleles seemed to be more frequent in the natural populations. The loss of

rare alleles may not represent a problem for gene conservation, as the rare alleles are usually the result of deleterious mutations or may be evolutionary relics (LINDGREN & GREGORIUS 1976).

Our results enable us to hypothesize that, although man-made, the Yatir afforestation project can be looked upon as a peripheral population, Sensu SAFRIEL *et al.* (1994), characterized by enhanced genetic diversity and, hence, improved resistance to extreme conditions. Therefore, the Yatir forest should be treated as a biogenetic resource used for rehabilitation and restoration of damaged Aleppo pine ecosystems.

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REFERENCES

CAVALLI-SFORZA, L. L. & EDWARDS, A. W. F. 1967: Phylogenetic analysis: Models and estimation procedures. *Evolution* **21**: 550-570.

CONDER, C. R. & KITCHENER, H. H., 1880: Map of Western Palestine, in 26 sheets, from the surveys conducted for the Committee of the Palestine Exploration Fund, Palestine Exploration Fund, London.

CONKLE, M. T., HODGSKISS, P. D, NUNNALLY B. L. & HUNTER, S. C. 1982: Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, General Technical Repport PSW-64.

EL-KASSABY, Y. A. & RITLAND, K. 1996: Impact of selection and breeding on the genetic diversity in Douglas-fir. *Biodiversity and Conservation* **5**: 795-813.

EL-KASSABY, Y. A., MEAGHER, M. D., PARKINSON, J. & PORTLOCK, F. 1987: Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia. *Heredity* **58**: 1973-1981.

EMBERGER, L., GAUSSEN, H., KASSAS, A. & DE PHILIPIS, A. 1963: Ecological study of the Mediterranean zone. Bioclimatic map of the Mediterranean zone. *Arid Zone Research*, UNESCO-FAO, Paris. 58 pp.

FURNIER, G. R. & ADAMS, W. T. 1986: Geographic patterns of allozyme variation in Jeffery pine. *American Journal of Botany* **73**: 1009-1015.

GONCHARENKO, G. G., PADUTOV, V. E. & SILIN, A. E. 1993: Allozyme variation in natural populations of Eurasian pines. I. Population structure genetic variation and differentiation in *Pinus pumila* (Pall.) Regel from Chukotsk and Sakhalin. *Silvae Genetica* **42**: 246-253.

GONCHARENKO, G. G., SILIN, A. E. & PADUTOV, V. E. 1994: Eurasian pines III. Population structure, di.fferentiation and gene flow in central and isolated populations of *Pinus sylvestris* L. in Eastern Europe and Siberia. *Silvae Genetica* **43**: 119-132.

GRUNWALD, C., SCHILLER, G. & CONKLE, M. T. 1986: Isozyme variation among native stands and plantations of Aleppo pine in Israel. *Israel Journal of Botany* **35**: 161-174.

GURIES, R. P. & LEDIG, F. T. 1981: Genetic structure of populations and differentiation in forest trees. pp. 42-47. *In:* Proceedings of the Symposium on Isozymes of North American Forest Tree and Forest Insects. (ed. M.T. Conkle), USDA For. Serv. Gen. Tech. Rep. PSW-48. pp. 42-48.

HAMRICK, J. L. & GODT, M. J. W. 1989: Allozyme diversity in plant species. *In*: Plant Population Genetics, Breeding and Genetic Resources. (eds. A.H.D. Brown, M.T. Clegg, A.L. Kahler, & B.S. Weir). pp. 43-63. Sinauer Associates, Inc., Sunderland, Massachusetts..

HAMRICK, J. L., BLANTON, H. M. & HAMRICK, K. J. 1989: Genetic structure of geographically marginal populations of ponderosa pine. *American Journal of Botany* **76**: 1959-1968.

KARA, N., KOROL, L., IŞIK, K. & SCHILLER, G. 1997: Genetic diversity in *Pinus brutia* Ten: Altitudinal variation. *Silvae Genetica* **46**: 155-161.

KOROL, L. & SCHILLER, G. 1996: Relations between native Israeli and Jordanian Aleppo pine (*Pinus halepensis* Mill.) based on allozyme analysis: a note. *Forest Genetics* **3**: 197-202.

LERNER, I. M. 1954: Genetic Homeostasis. Wiley, New York.

LINDGREN, D. & GREGORIOUS, H. 1976: Inbreeding and coancestry. *In:* Proceedings IUFRO Joint Meeting on Advanced Genetic Breeding, Bordeaux, France, pp 49-71.

LINHART, Y. B., MITTON, J. B., STURGEON, K. B. & DAVIS, M. L 1981: Genetic variation in space and time in a population of ponderosa pine. *Heredity* **46**: 407- 426.

MELZACK, R. N., GRUNWALD, C. & SCHILLER, G. 1981: Morphological variation of Aleppo pine (*Pinus halepensis* Mill.) in Israel. *Israel Journal of Botany* **30**: 199-205.

MELZACK, R.N., SCHILLER, G. & GRUNWALD, C. 1982: Seed size, germination, and seedling growth in *Pinus halepensis* Mill. and their relation to provenances in Israel. Leaflet, Department of Forestry, Agricultural Research Organization, Ilanot No. 72.

MORANDINI, R. 1976: Mediterranean conifers. *Forest Genetic Resources, Information No. 5; Forestry Occasional Paper* 1976/1, FAO, Rome, pp. 12-18.

MÜLLER-STARCK, G. 1985: Genetic variation under extreme environmental conditions. *In:* Population Genetics and Genetic Conservation of Forest Trees. (eds. Ph. Baradat, W.T. Adams & G. Müller-Starck). pp. 201-210. SPB Acad. Pub. Amsterdam.

NAHAL, I. 1981: The Mediterranean climate from a biological viewpoint. Mediterranean-Type Shrublands. (eds, F. Di Casrti, D. W. Coodall, & R. L. Specht) Ecosystems of the World **3**, 63-86. Elsevier, Amsterdam.

NEI, M. 1973: Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. U.S.A.* **70**: 3321-3323.

NEI, M. 1977: F-statistics and analysis of gene diversity in subdivided populations. *Ann. Human Genetics.* **41**: 225-233.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number if individuals. *Genetics* **89**: 583-590.

NEVO, E. 1983: Population genetics and ecology: the interface. Evolution from Molecules to Men. (ed. D.S. Behdall), pp. 223-272. Cambridge University Press, Cambridge, UK.

NEVO, E. 1997: Evolution in action across phylogeny caused by microclimatic stress at "Evolution Canyon". *Theoretical Population Biology* **52**: 231-243.

NEVO, E. 1998: Molecular evolution and ecological stress at global, regional and local scales: The Israeli perspective. *Journal of Experimental Zoology* **282**: 95-119.

OPPENHEIMER, H. R. 1967: Mechanism of Drought Resistance in Conifers of the Mediterranean Zone and the Arid West of the U.S.A. Final report on Project No A10-FS, Grant No FG-Is: 19. The Hebrew University of Jerusalem.

PANETSOS, C. P. 1981: A monograph of *Pinus halepensis* Mill and *P. brutia* Ten. *Annals of Forestry,* Zagreb **9**: 39-77.

SAFRIEL, N. U., VOLIS, S. & KARK, S. 1994: Core and peripheral populations and global climate change. *Israel Journal of Plant Sciences* **42**: 331-345.

SCALTSOYIANNES, A., ROHR, R., PANETSOS, K. P. & TASAKTSIRA, M. 1994: Allozyme frequency distributions in five European populations of black pine (*Pinus nigra* Arnold). *Silvae Genetica* **43**: 20-30.

SCHILLER, G. 2000: Inter- and Intra specific genetic diversity of *Pinus halepensis* Mill. and *P. brutia* Ten. *In:* Ecology and Biogeography and Management of *Pinus halepensis and P. brutia* Forest Ecosystems in the Mediterranean Basin. (eds. G. Ne'eman, & L. Trabaud), pp. 13-35, Backhuys Publishers, Leiden.

SCHILLER, G. & WAISEL, Y. 1989: Among-provenance variation in *Pinus halepensis* in Israel. *Forest Ecology and Management* **28**: 141-151.

SCHILLER, G., CONKLE, M. T. & GRUNWALD, C. 1986: Local differentiation among Mediterranean populations of Aleppo pine in their isoenzymes. *Silvae Genetica* **35**: 11-18.

SCHILLER, G., KOROL, L., UNGER, E. D., ZEHAVI, A., GIL, S. L. & CLIMENT, M. J. 1999: Canary Island pine (*Pinus canariensis* Chr. Sm. Ex. DC.) 1. Differentiation among native populations in their isoenzymes. *Forest Genetics* **6**: 257-276.

SOULE, M. E. 1979: Heterozygosity and developmental stability: another look. *Evolution* **33**: 396-401.

SWOFFORD, L. D. & SELANDER, B. C. 1981: BIOSYS-1, A Computer Program for the Analysis of Allelic Variation in Genetics. Users Manual. Department of Genetics and Development. University of Illinois at Urbana-Champaign. Urbana, Illinois 60801, USA

WRIGHT, S. 1965: The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**: 395-420.