HABITAT EFFECTS ON ADAPTIVE GENETIC VARIATION IN *PINUS HALEPENSIS* MILL. PROVENANCES[1]

Gabriel Schiller[a] , Leonid Korol & Galina Shklar

Corresponding author: Department of Agronomy and Natural Resources, Forestry Section, Agricultural Research Organization, the Volcani Center, P.O.Box 6, 50250 Bet Dagan, Israel. e-mail: vcgabi@volcani. agri. gov.il, Tel : +972-3-1683683

SUMMARY

The nature of allozyme differentiation among Aleppo pine (*Pinus halepensis* Mill.) circum-Mediterranean populations gave rise to the division of the species into two major subdivisions and several races. In spite of the relatively low values of several genetic parameters in *Pinus halepensis,* compared with the values of the same parameters in other pine species, there is enough genetic variation to enable selection and adaptation to stressful environments, within and outside the natural range of distribution of the species.

Aleppo pine F_1-offspring, grown from seeds collected in natural native Israeli and overseas populations, were planted in 1985 in two provenance trials. One of the trials was established under drought-prone environmental conditions at the northern edge of the Israeli Negev Desert (the Yatir Forest), to mimic the ecological changes resulting from global climate change.

We tested whether these 13-year-old F_1-offspring populations exhibited genetic diversity and structure similar to those of their parental origin populations. We also measured several eco-physiological properties, to elucidate eventual differences between provenances in their response to drought.

After 13 years, in 1997, the survival rate of the 23 provenances averaged 75 ± 13 %, and ranged between 46 and 92 %. The survival rate in 2000 declined to an average of 38 ± 21 %, with a range from 0.0 to 92 %; this was because of two successive winter droughts, with only 144 and 155 mm rainfall in the winters of 1998/99 and 1999/2000, respectively. This was the most severe such event ever recorded in the region.

The genetic diversity of the provenances was determined in 1997/1998 by means of isoenzyme analysis by starch gel electrophoresis, and by means of genome DNA analysis by RAPD-based PCR. The percentage of polymorphic loci increased from an average of 40 % in the natural populations to 46 % in the F_1-offspring populations at Yatir. The average number of alleles per locus (*A/L*) rose from 1.3 in the parental populations to 1.5 in the offspring populations and, consequently, the observed heterozygosity rose from 0.049 and 0.115 respectively in the parental populations to 0.160 and 0.144, respectively, in the Yatir populations. 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 relatively minor changes occurred in those from relatively drier environments. Analysis by means of RAPD-based PCR showed that gene diversity (h) rose from 0.264 and 0.303, respectively, in the parental natural populations to 0.431 and 0.406 in Yatir. The calculated linear regression between allozyme heterozygosity and gene diversity (RAPD) resulted in $r = 0.690$, $P < 0.001$.

Planting in dryland areas, outside the natural area of distribution of a species, can be looked upon as the creation of man-made peripheral populations as defined by SAFRIEL *et al.* (1994). Such populations will have higher genetic diversity than natural populations – conferred by selection pressure that eliminates the homozygous and favors the heterozygous genomes – and consequently have more resistance to extreme conditions. Therefore, plantations of such populations should be established and treated as a biogenetic resource, available for rehabilitation and restoration of damaged natural or planted Aleppo pine ecosystems.

INTRODUCTION

Forest species are subjected to numerous environmental stresses; most of which influence the plant's water status. Water is very significant because of its biological roles: as a solvent and a transport medium; as an electron donor in biochemical reactions; and as an evaporative coolant. Tree water balance is

[1] This paper has been presented at the IUFRO Symposium on Population and Evolutionary Genetics of Forest Trees held in Stará Lesná, Slovakia, on August 25–29, 2002.

Contribution number 104/2003 of the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

very often impaired by the stresses imposed by external drought conditions, which affect forest trees across all geographical and climatic areas. Species of woody plants are known to vary in their sensitivity to increase in soil-water potential, an important factor that is required for normal growth processes. It is assumed that all plants have an encoded capability to respond to this stress. Although many plant species have developed responses and adaptations that are specific to a particular stress such as drought, and are known to be genetically controlled, not every plant species is capable of such an adaptation. Within species whose genotypes have the capability to adapt, the most resistant genotypes in a population may be found at the edges of its habitat, where the environmental stresses are strongest. Genetic differentiations possibly result from divergent evolutionary forces such as selection and genetic drift. Gene flow influences the genetic differentiation of species and populations within species. High rates of gene flow among populations smooth the effects of genetic drift and selection. These effects create new gene combinations, which help the species to adapt to new conditions, i.e., microgeographic differentiation (WRIGHT 1951; CROW & AOKI 1984; SLATKIN 1985 1987).

The genus *Pinus* includes about 100 species and is among the most widespread tree genera in the Northern Hemisphere. Pines are of major ecological and economic significance. Among the lowland Mediterranean pine species Aleppo pine (*Pinus halepensis* Mill.) is the most widely spread, and the most drought resistant (OPPENHEIMER 1967, SHOMER-ILAN 1968).

Aleppo pine seed material from various circum-Mediterranean geographic regions became available in 1976 (MORANDINI 1976), 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 morphological and genetic diversity of circum-Mediterranean and local Aleppo pine populations (SCHILLER 2000).

Geographic trends of allozyme differentiation among Aleppo pine populations gave rise to the division of the species into two major groups: a western Mediterranean group that occupies a region extending from longitude 25° 00' E westward toward the Atlantic; and an eastern Mediterranean group in the region from longitude 25° 00' E eastward, into the Middle East and Turkey. Furthermore, genetic parameters indicated that the western Mediterranean group consists of four geographic races: (i) western Europe, (ii) eastern Europe, (iii) Morocco and (iv) North Africa (SCHILLER *et al.* 1986), a more recent study (KOROL *et al.* 2001) yielded similar results. In spite of the relatively low values of several genetic parameters (*e.g.*, number of polymorphic loci, number of alleles per polymorphic locus, direct count and expected heterozygosity) in comparison with those found in other pine species, there is still enough genetic variation to enable the species to adapt to stressful environments outside its natural area of distribution.

Afforestation activity outside the natural climatic range of the various species has created peripheral populations – as defined by SAFRIEL *et al.* (1994) – of some of the most important Mediterranean conifer species, such as *Pinus halepensis, P. brutia, P. eldarica, P. pinea, P. canariensis, Cupresus sempervirens*, etc. These populations have lived for more than 20 years under some highly stressful climatic conditions, in comparison with those prevailing at the core area of their natural distribution. These very stressful and variable conditions could induce selection that contributes to and maintains a very large genetic diversity.

Genetic diversity and structure were studied in a 36-year-old Aleppo pine afforestation at Yatir, a drought-prone area at the northern edge of the Israeli Negev Desert (31° 02' N, 35° 02' E, 630–700 m a.s.l.). This region is characterized by higher solar radiation and lower atmospheric relative humidity than those in regions in which natural Aleppo pine forests and small relicts grow in Israel; and only 278 (±89) mm 35-year average annual rainfall (Subdesertic climate), in comparison with more than 500 mm in regions in which natural Aleppo pine forests and small relicts grow in Israel. The percentage of polymorphic loci and the direct-count mean heterozygosity were 52 and 17.8 %, respectively, compared with 49.6 and 14.9 %, respectively, in the native Israeli populations. From these results it can be deduced that the Yatir forest is under a selection pressure that only more heterozygous individuals are able to withstand. Many studies have found that severe environmental conditions may tend to select for a more heterozygous population, for example: *Ips pini* (GAST & STOCK 1994); *Gambusia holbrooki* (MULVAY *et al.* 1994); *Triticum dicoccoides* (LI (Y.C.) *et al.* 2000); in European Scots pine populations (PRUS-GŁOWACKI *et al.* 1999); *Hordeum spontaneum* (VOLIS *et al.* 1998); and in wild barley and chukar partridge (SAFRIEL *et al.* 1994).

The aim of the present study was to investigate whether changes have occurred in genetic parameters of several provenances as the result of adaptation to stressful ecological conditions that partially

mimic the ecological changes that may eventually result from global climate change.

For this study we have used genetic material from a provenance trial that was planted in 1985 within the Yatir afforestation project with seeds that were collected in 1976 and 1980 in the natural populations (= parental populations). Thirteen years after planting of that provenance trial, the average survival rate was 75 ± 13 %; after a further three years, which included two years of very severe drought, with only 144 and 150 mm annual rainfall, respectively, the average survival rate had declined to 38 ± 21 %. The existence, at the edge of the desert, of that provenance trial planted with 23 F_1-offspring populations, enabled us to quantify changes at the allozyme and DNA levels, within provenances, among populations (planted versus natural origin) in response to 13 years of environmental stress conditions.

MATERIALS AND METHODS

The seed material analyzed

Genetic diversity and structure were analyzed in seed material received from:

a. INRA at Avignon, France, Department of Forest Trees Population Genetics, seed collection, in connection with the European Communities International Cooperation with Developing Countries, Contract No: ERBIC 18CT 970 200.

b. Several F_1-progeny artificial populations growing since 1985 in the Yatir forest provenance trial.

In the summer of 1997 cones were collected in each of the parental populations growing in Israel, as far as possible from the same trees as in 1980, and from their F_1-offspring artificial populations in the Yatir forest. Open-pollinated (half-sib) seeds were extracted from the cones and those from each tree were kept separately. In addition, seed lots were received from many overseas Aleppo pine populations, some of them from exactly the same populations and geographic locations that were involved in the 1976 seed collection (MORANDINI 1976). The populations analyzed are listed in Table 1.

Isoenzyme marker analysis

For measuring genetic variability parameters in the populations, 14 enzyme systems were used: *Aap; Aco; Acp; Adh-1, -2; Cat; Gdh; Got-1, -2, -3; Idh; Lap; Mdh, -1, -2, -3, -4; Mnr-1,-2; Pgi-1, -2; Pgm-1,-2; 6pgd-2,-3* (for details see KOROL *et al.* 2002). Seed

Table 1. Geographic locations of natural populations analyzed.

Seed source		Country and FAO/IUFRO code		Longitude	Latitude	Altitude (m a.s.l.)
1	Tamga	Mor-1	–	6° 07' W	32° 02'	1350
2	Ikherifene	Mor-2	–	4° 35' W	35° 07'	915
3	Lalla Mimouna	Mor-3	–	3° 07' W	34° 03'	110
4	Selloum	Tun-1	A-10	8° 40'	35° 05'	–
5	Birino	Tun-2	–	8° 37'	35° 28'	950
6	Oum Jedour	Tun-3	A-9	8° 57'	35° 38'	–
7	Takrouna	Tun-4	–	–	–	–
8	Telagh*	Alg	A-31	–	–	–
9	Velez Blanco	Spa-1	A-18	2° 10' W	37° 40'	1200
10	Jarafuel	Spa-2	A-20	1° 00' W	38° 55'	600
11	Caireval	Fra	–	5° 21'	43° 40'	300
12	Chiavari	Ita-1	–	9° 19'	44° 19'	–
13	Otricoli*	Ita	A-26	12° 38'	42° 24'	400
14	Vico del Gargano*	Ita-2	A-27	16° 00'	41° 54'	225
15	Istiaia (Ebouea)*	Gre-1	A-3	23° 17'	38° 56'	125
16	Kassandra*	Gre-2	A-5	23° 28'	40° 02'	60
17	Jerash	Jor-1	–	35° 54'	32° 17'	–
18	Ajlun	Jor-2	–	35° 46'	32° 20'	–
19	Yirka	IL-2	–	35° 10'	32° 57'	430
20	Bet J'ann*	IL	–	35° 23'	32° 53'	850
21	Mt. Carmel*	IL-1	–	35° 02'	32° 43'	450
22	Turkey	Tur	Seed orchard	–	–	–

* = F_1-offspring of these natural forests are planted in the Yatir provenance trial.

preparation, germination, enzyme extraction and horizontal starch gel electrophoresis, gel slicing and staining were performed according to CONKLE *et al.* (1982), with several adjustments according to KOROL & SCHILLER (1996). To raise the probability of detecting a heterozygous tree, analyses were performed with eight haploid megagametophytes per mother tree ($P = 0.992$)

RAPD marker analysis

For RAPD analysis six 10-base primers (from sets OPA OPB and OPD, Operon, Alameda, USA) were used. Genomic DNA was extracted from the megagametophytes by a modified CTAB procedure (DOYLE & DOYLE 1990), and the DNA concentration of each sample was measured by spectrophotometric assay at 260 nm.

PCR was performed in a 15-µl sample of reaction solution (KOROL *et al.* 2002). The following amplification parameters were used: denaturation step at 95 °C for 2 min; 44 cycles of 94 °C for 10 sec, 37 °C for 1 min and 72 °C for 2 min; and a final step of 72 °C for 5 min. The products of the amplification were electrophoresed in a 1.8% agarose gel, followed by ethidium bromide staining (SAMBROOK *et al.* 1989). The molecular sizes of the RAPD products were estimated by comparison with molecular weight markers. After electrophoresis, the gels were photographed, and the photographs were used to score the data for the RAPD analysis. The RAPD specific amplification products were scored as: present = 1, absent = 0. The value 1 indicated the presence of a certain fragment, and 0 its absence.

Data analysis

For the allozyme analyses the calculations of intra- and inter-population genetic diversity were done with the BIOSYS-1 computer program, version 1.7 (SWOFFORD & SELANDER 1981). The scored allele frequency data were used for calculation of the proportion of polymorphic loci (*P%*), the mean number of alleles per locus (*A/L*), and observed (*Hobs*) and expected heterozygosity (*Hexp*). The unbiased values were computed according to conditional expectations in NEI (1978). Wright's Fst expectation (WRIGHT 1965; NEI 1977) was used to analyze the genetic differentiation between populations. Inbreeding coefficients were summarized across populations for each locus by calculation Fis values. The significance of the excess and/or deficiency in heterozygotes was tested with Li's formula (LI & HOROVITZ 1953; NEI 1977).

The POPGEN computer program version 1.32 (YEH *et al.* 1999) was used to estimate the genetic polymorphism at the nucleotide level. RAPD data were analyzed to obtain the mean number of alleles per locus (*na*), the expected heterozygosity (*h*) according to NEI (1973) and the Shannon index (*I*) introduced by LEWONTIN (1972).

The genetic distances between populations were computed by treating every locus according to CAVALLI-SFORZA & EDWARDS (1967). Cluster analyses were performed with PHYLIP (Phylogeny Inference Package) ver. 3.5 (FELSENSTEIN 1993). Identification of natural groups was based on the allozyme marker data by using the neighbor-joining method of clustering (SAITOU & NEI 1987). All trees were constructed by successive clustering of lineages, setting branch lengths as the lineages joined.

For multiple comparisons of differences among populations and among groups, the analysis of variance (ANOVA) algorithm with SIGMASTAT STATISTICAL software (SPSS Inc.) was used

RESULTS

Differentiation among circum-Mediterranean populations

A dendrogram based on genetic distance measures (CAVALLI-SFORZA & EDWARDS 1967) obtained with the Neighbor-joining algorithm is presented in Figure 1. The phylogenetic tree shows a distinct separation among groups. The four groups corresponded to the geographic distribution of the populations in the Mediterranean region:

1. Western European including Morocco, Spain and France;
2. Eastern European including Italy and Greece;
3. The four populations growing in Tunisia; and
4. Eastern Mediterranean including Israel, Jordan and Turkey.

Differences among the four groups were found in most of the loci, with significant differences in the *Aap, Aco, Adh2, Cat2, Got3, Lap1, Mdh2, Mdh4, Mnr1, Pgm2* and *6Pgd3* loci. In comparison with the others three groups the fourth group (eastern Mediterranean group) had high frequencies of the second allele in the *Aap, Cat2* and *Got3* loci. The second group (Greek-Italian group) differed from the others mainly in allele frequencies in the *Aap, Aco, Adh1, Adh2, Gdh, Lap, Mdh4, Pgi2*, and *6Pgd2* loci; in many enzyme systems the third and fourth alleles were present only in the Greek-Italian populations.

The same four groups could also be established

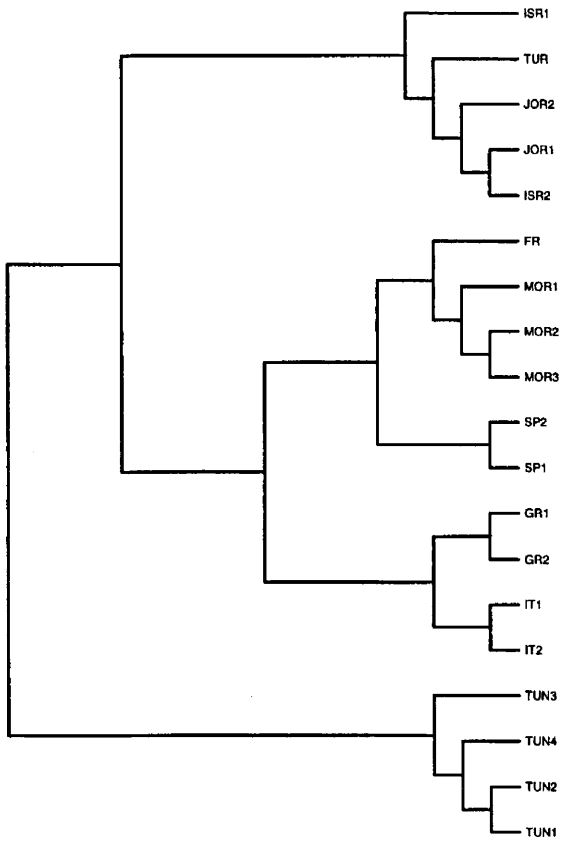

Figure 1. Dendrogram for 19 Mediterranean *Pinus halepensis* populations based on CAVALLI-SFORZA and EDWARDS (1967) distance, produced by the Neighbor-joining method (SAITOU & NEI, 1987). (Populations according to table 1).

on the basis of some genotype frequencies, as presented in Figure 2. The figure demonstrates the geographic distribution of genetic types, as esti mated according to their frequencies. The Aap locus clearly indicates differences among the groups. The *Aap 1-1* genotype was the most frequent one in the first group, with an average frequency of 0.965, whereas in the other groups its mean frequency did not exceed 0.450. This genotype frequency in the first group differed significantly ($P < 0.001$) from those in the other three groups. The *Aap 2-2* genotype was the most frequent (0.452) in group four, and its frequency there differed significantly from those in group one ($P < 0.0001$), group two ($P < 0.054$) and group three ($P < 0.161$). The *Aap 3-3* genotype was detected only in the second (the Greek-Italian) group, where its average frequency was 0.117; this genotype was never detected in trees from other regions. High frequencies of the *Adh1 1-2* and the *Lap 2-2* genotypes also characterized the Greek-Italian group. The *Lap 2-2* and the *Aap 3-3* genotypes were detected only in the second group.

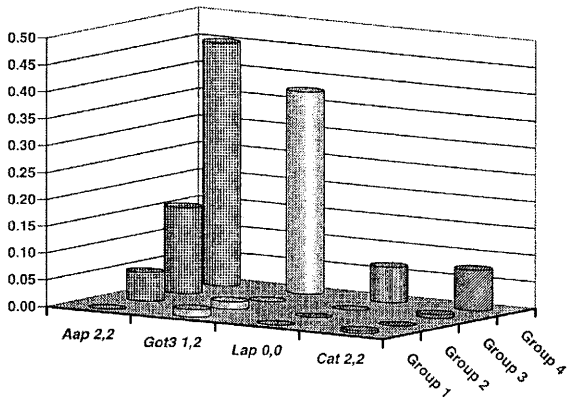

Figure 2. Frequencies of allozyme genotypes in the four Aleppo pine geographic groups of the Mediterranean region.

The *Lap 0-1* genotype was found only in the fourth group, being very frequent in the Israeli populations. The *Cat2 1-2* genotype was the most frequent in both the eastern Mediterranean and the Tunisian populations, whereas it was not detected in the other groups.

Values of genetic diversity parameters could also be used to establish these four groups as presented in Table 2. The western European and Moroccan group showed the lowest degree of within-population diversity. Distribution of the mean numbers of alleles per locus in groups ranged between 1.2 and 1.4 in the first group, between 1.8 and 2.0 in the second group, between 1.5 and 1.6 in the third group, and between 1.3 and 1.8 in the fourth group. ANOVA analysis indicated significant differences ($P < 0.05$) in mean values of number of alleles per locus between the eastern European group and the other groups. The percentages of polymorphic loci according to the 95 % criterion were between 16 and 20 % in the first group, between 44 and 52 % in the second group, between 32 and 36 % in the third group, and between 12 and 44 % in the fourth group.

Of the four groups, the eastern European group displayed the highest level of mean heterozygosity, 0.183, whereas the second and third groups showed considerably lower variation (0.103, 0.124), and the first group (western European) displayed the lowest value of expected heterozygosity ($H_e = 0.059$). Pairwise comparisons of genetic variation between geographic groups revealed significant differences of heterozygosity ($P < 0.05$): group1 vs. group 2; group 2 vs. group 3; group 2 vs. group 4 and group 4 vs. group 1.

Differentiation among microenvironmental sites

Genetic diversity in sites was analyzed by means of isoenzyme electrophoresis and by the RAPD tech-

Table 2. Genetic variability at 25 loci in 20 circum-Mediterranean populations of *Pinus halepensis* Mill.

Population	*N*	*All/L*	*P%*	Mean heterozygosity	
				H_o^*	H_e^{**}
Tamga	17	1.3±0.1	20.0	0.049±0.028	0.067±0.027
Ikherifene	34	1.4±0.1	20.0	0.036±0.017	0.068±0.024
Lalla Mimouna	16	1.2±0.1	20.0	0.025±0.013	0.052±0.024
Velez Blanco	36	1.3±0.1	20.0	0.051±0.028	0.055±0.024
Jarafuel	72	1.4±0.1	16.0	0.045±0.021	0.059±0.024
Caireval	43	1.4±0.1	20.0	0.042±0.020	0.054±0.022
Chiavari	108	1.8±0.1	44.0	0.137±0.039	0.186±0.046
Vico del Gargano	108	1.8±0.2	44.0	0.144±0.067	0.180±0.043
Istiaia	76	2.0±0.2	52.0	0.138±0.036	0.188±0.041
Kassandra	45	1.9±0.2	48.0	0.126±0.037	0.177±0.038
Selloum	43	1.5±0.1	32.0	0.085±0.033	0.111±0.034
Birino	72	1.5±0.1	32.0	0.059±0.250	0.076±0.025
Oum Jedour	65	1.5±0.1	36.0	0.059±0.021	0.097±0.027
Takrouna	57	1.6±0.1	36.0	0.088±0.030	0.129±0.036
Jerash	167	1.6±0.1	44.0	0.110±0.039	0.156±0.039
Ajlum	54	1.4±0.1	32.0	0.121±0.045	0.123±0.037
Yirka	37	1.5±0.1	36.0	0.124±0.042	0.118±0.035
Bet J'ann	27	1.3±0.1	12.0	0.049±0.024	0.054±0.027
Mt. Carmel	238	1.8±0.1	44.0	0.116±0.029	0.142±0.033
Turky	30	1.4±0.1	44.0	0.103±0.033	0.151±0.038

N = mean sample size per locus,
All/L = mean number of alleles per locus,
P% = percentage of polymorphic loci,
H_o = observed heterozygosity,
H_e = expected level of heterozygosity,
* = a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95,
** = unbiased estimate (see NEI 1978).

nique in several F_1-progeny populations growing in the provenance trial at Yatir (see footnote to Table 1).

Calculation of the linear regression between the F_1-offspring survival rate (independent variable) and the increase in the percentages of observed heterozygosity, or in the number of heterozygous genotypes per tree for each provenance (dependent variables) resulted in negative correlation coefficients of –0.9992 and –0.9978, respectively ($P < 0.05$). The correlation coefficient of the linear regression between F_1-offspring survival rate and the increase of F_{is} values was positive 0.9733 ($P < 0.05$).

Within-provenance, among-sites (progeny planted at Yatir versus natural parental) differences in allele frequencies were detected in almost all enzyme systems analyzed. Frequencies of several alleles in the loci *Mdh-3*, *Mdh-4* and *Pgm-2* were higher in the F_1- progenies than in the parental natural populations; in the *Mdh-4* and *Pgm-2* loci the differences were related to the alteration of heterozygous genotypes. In the *Lap* and *Mdh-3* loci changes were related to more frequent homozygous genotypes. The mean frequency of allele 1 in the *Aap* locus among 20 circum-Mediterranean populations was 0.299 (KOROL *et al.* 2001) for non-Israeli provenances it was 0.231 and for the Israeli provenances alone, –0.716. Differences between parental natural populations and their F_1-progeny in the Yatir forest were found in the common allele "1" of the *Pgm-2* locus. Similar differences were found in the null allele of the *Mdh-3* locus. The frequency of this null allele in the *Mdh-3* locus was always higher in the F_1-progeny in Yatir than in their parental natural populations.

Table 3a presents genetic variability parameters in several F_1-progeny populations in comparison with these parameters in the parental natural populations. Percentages of polymorphic loci (*P %*), observed heterozygosity (H_o) and expected

Table 3a. Genetic variability parameters at the allelic level in some *Pinus halepensis* populations.

Population	*N*	*A/L*	*P%*	H_O	H_E
Telagh (Yatir)	5	1.6	52	0.216	0.221
Telagh (Natural)	–	–	–	–	–
Istiaia (Yatir)	2676	1.9	6052	0.220	0.214
Istiaia (Natural)		2.2		0.138	0.188
Otricoli (Yatir)	22	1.6	36	0.145	0.152
Otricoli (Natural)	–	–	–	–	–
Bet J'ann (Yatir)	1627	1.5	4012	0.160	0.133
Bet J'ann (Natural)		1.3		0.049	0.054
Mt. Carmel (Yatir)	18238	1.7	4844	0.144	0.153
Mt. Carmel (Natural)		1.8		0.116	0.152

N = mean sample size per locus,
A/L = mean number of alleles per locus,
P% = percentage of polymorphic loci,
H_o = observed heterozygosity,
H_e = expected level of heterozygosity,
* = a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95,
** = unbiased estimate (see NEI 1978).

Table 3b. Genetic diversity parameters at the nucleotide level in some *Pinus halepensis* populations growing in different sites.

Population	Site	*N*	n_a*	*h**	*I**
Yirka	natural	31	2.0	0.316	0.476
Bet J'ann	natural	20	1.8	0.264	0.405
Mt. Carmel	natural	27	1.9	0.303	0.452.
Bet J'ann	Yatir	14	2.0	0.431	0.619
Mt. Carmel	Yatir	14	2.0	0.406	0.589

* n_a = Observed number of alleles, *h* = NEI's (1973) gene diversity, *I* = Shannon's Information Index [LEWONTIN, 1972]. In comparison with *h*, *I* is unbounded, varying to a maximum value of ln(*a*)

heterozygosity (H_e) were higher in the progeny populations at Yatir than in the natural populations. Expected heterozygosity values in 15 of the 23 loci (65 %) detected in the provenances growing in the Yatir forest provenance trial were larger than those in the same loci in the parental natural forests. T-test analysis showed that the differences between the two groups were statistically significant ($P = 0.0135$).

Table 3b shows the ranges of gene diversity (*h*) – between 0.264 and 0.431 – and of Shannon's index (*I*) – between 0.405 and 0.619. All genetic estimates of *h* and *I* were higher in the Yatir forest than in the natural populations. Estimations of both allozyme and RAPD heterozygosity showed increases, with linear regression coefficient $r = 0.690$, $P < 0.001$, n = 5. Estimates of genetic diversity based on Shannon's index were similar and the Yatir populations showed a higher level of diversity. There were no site-specific RAPD markers for the Yatir Forest populations.

Excess or deficiency of heterozygotes for each locus and for each population were examined by means of Wright's Fixation Index, i.e., the "inbreeding coefficient". The results presented in Table 4 show different heterozygote frequencies in each population. However, when F_{is} values were pooled according to location (natural versus Yatir), two distinct groups emerged. Within the Yatir group (F_1-progeny populations) most of the F_{is} values were lower than zero, which expresses their excess of

Table 4. Fixation indices of polymorphic loci in several analyzed populations.

Population / Locus	Istiaia		Bet J'ann		Mt. Carmel	
	Natural	Yatir	Natural	Yatir	Natural	Yatir
Aap	0.141	0.218	0.047	–0.103	0.045	0.438
Aco	0.108	0.008	–	0.238	–0.067	–0.200
Acp	–0.056	–0.268	–	–	–	–0.205
Adh-1	–0.005	–0.182	–	–	–	–0.241
Adh-2	0.857	–0.033	–0.080	–0.103	0.214	–0.059
Cat-2	–0.007	–0.040	0.181	–0.524	–0.158	–
Gdh	–	–0.130	–	–0.333	–0.008	–
Got-1	–	–0.020	–	–	–	–
Got-2	–	–	–	–	–0.056	–
Got-3	–	–0.106	–	–	–0.057	–
Idh	0.551	–	–	–0.032	–	–
Lap-1	0.516	–0.048	–0.038	–0.103	–0.013	–0.043
Mdh-1	–0.007	–	–	–	–	–
Mdh-2	1.000	–	–0.038	–	0.659	–0.091
Mdh-3	1.000	–0.038	–0.019	–0.231	0.495	0.500
Mdh-4	0.394	–0.529	–0.019	–0.391	–0.200	–0.286
Mnr-1	–0.056	0.188	–	–0.524	–0.246	–0.125
Mnr-2	–	–0.020	–	–	–	–0.029
Pgm-2	0.789	0.175	–	–0.067	–0.199	–0.026
Pgi-1	–	–0.040	–	–	–	–
Pgi-2	0.165	–0.061	–	–	–0.048	–0.029
Pgd-2	–0.188	–0.182	–	–	–	–
Pgd-3	–0.065	0.114	–	–0.032	–0.008	–0.091
Mean F_{IS}	0.256	–0.052	0.005	–0.184	0.033	–0.035

heterozygotes. Within the second group (parental natural populations) most F_{is} values were positive, which reflects a deficiency in heterozygotes. Comparison between the F_{is} of these two groups showed a statistically significant P value of 0.0017. The mean estimated F_{is} of the natural population was 0.098 and that for the Yatir populations –0.090, the difference between the two means reaching 0.188. The differences in the within-pair fixation indexes, i.e., natural populations versus their F_1-offsprings, were statistically significant for two of the three provenances – Istiaia and Bet J'ann – with P values of 0.0131 and 0.0199, respectively.

DISCUSSION

Forest trees are a valuable asset worldwide and play an important role in our global environment. Processes of tolerance of and/or adaptation to habitats are difficult to explain without studying genetic multiplicity. The environment may strongly affect and change the genetic variation and create favorable conditions for natural selection.

In three of the 14 enzyme systems analyzed, differences in allele frequencies were revealed between the parental natural populations and their F_1-offspring growing in the Yatir forest provenance trial. In these enzyme systems there are loci in which a certain allele is probably more sensitive than others to stressful conditions. Increased frequency of allele 2 in the *Aap* locus characterizes the east-Mediterranean group (SCHILLER *et al.* 1986) Hence, it is reasonable to assume that the increased frequency of allele 2 in the *Aap* loci was related to adaptation to environmental conditions.

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 we prefer natural selection, that favors heterozygotes, and consequently increases the heterozygosity as the populations adapt to the environment.

Genetic diversity in nature is the result of evolutionary processes, and it is apparent within species at different levels in both the enzymes and the DNA (NEVO 1998). Various authors have proposed adaptation to local environmental conditions as an explanation for differences in allele frequencies in geographically widely distributed forest tree species. It has been hypothesized that genetic differentiation within species are related to adaptation to micro-geographic changes within the area of distribution (LINHART *et al.* 1981; HAMRICK & GODT 1989). FURNIER & ADAMS (1986) found correlation between allele frequencies and adaptation to ultramafic soils, and GURIES and LEDIG (1981) found significant correlations between allele frequencies and climatic variables such as winter temperature. In the Swiss sub-alpine stands of *Picea abies* and *Fagus sylvatica* MÜLLER-STARCK (1995) found relatively large intra-populational and average inter-populational genetic variation in comparison with reference populations in Europe.

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).

According to MÜLLER-STARCK (1987) and BERGMANN & RUETZ (1991) the levels of genetic diversity in stressed plantations of forest tree species are similar to or higher than those in favorable natural populations. Similarly, in the present study, the numbers of heterozygous phenotypes present in natural populations were found to be considerably lower than those in the offspring populations in Yatir. Heterozygous individuals are believed to be more stable than their homozygous counterparts because of some inherently superior biochemical efficiency possessed by heterozygotes (LERNER 1954). Most of the trees in 20 provenances that survived under the ecological conditions of Yatir (an average of 44 % of the number planted) were heterozygous (unpublished data). These result gives rise to the hypothesis that as a population adapts to new climatic conditions increase of genetic variability may be expected and that the number of heterozygotes would correlate with environmental features

Allozymes and RAPD markers were used to analyze the genetic diversity within and between natural *Pinus halepensis* Mill. populations, and between them and their offspring populations, and to relate the intra-specific variability to the ecological differences among the sites (EL-KASSABY 1995). Comparisons between expected heterozygosity (allozyme markers) and gene diversity (RAPD markers) revealed a high probability that the variables were correlated, with a positive, high and significant correlation coefficient. Thus both methods showed an increase in hetrozygosity in populations growing in new environments. Increased emphasis has recently been placed on adaptive selection: NEVO (1997; 1998), in his "Evolution Canyon" studies, found correlations between environmental conditions and plant species diversity. This parallelism between diversity and microhabitats suggests that genetic and physiological diversity should be represented as arising from a complex of adaptive factors, related to environmental heterogeneity. Climatic natural selection through water and temperature stress appears to be a major differentiating factor (DANCIK & YEH 1983). The effects of climatic selection on individuals in the Yatir forest seemingly affected the heterozygosity.

Levels of genetic variation are presented in Tables 3a, 3b and 4. In general the values increased in the populations growing in the provenance trial in Yatir; in other words, genetic diversity is higher under stressful conditions (SAFRIEL *et al.* 1994). We consider that the correlation between gene diversity and survival rates, with negative correlation coefficients, suggests a model of heterozygosity in which heterozygous individuals are developmentally more stable than their homozygous counterparts (LERNER 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 to suppose 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 heterogeneity and structure within and between plant populations. Nevertheless, adaptive selection is receiving increasing attention: non-selective processes, which cause loss of allele heterozygosity, probably influence all components of population adaptation. 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.

Our results enable us to hypothesize that, although man-made, the Yatir afforestation project can be looked upon as a peripheral population, as defined by 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 bio-genetic resource, to be used for rehabilitation and restoration of damaged Aleppo pine ecosystems.

REFERENCES

BERGMANN, F. & RUETZ, W. 1991: Isozyme genetic variation and heterozygosity in random tree samples and selected orchard clones of the same Norway spruce populations. *For. Ecol. Manage.* **46**: 39–47.

CAVALLI-SFORZA, L. L. & EDWARDS, A. W. F. 1967: Phylogenetic analysis: models and estimation procedures. *Evolution* **32**: 550–570 (also *Amer. J. Human Genet.* **19**: 233–257).

CONKLE, M. T., HODGEKISS, 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, *Gen. Tech. Rep. PSW-64.*

CROW, J.F. & AOKI, K. 1984: Group selection for a polygenic behavioral trait, estimating the degree of population subdivision. *Proc. Natl. Acad. Sci. USA,* **81:** 6073–6077.

DANCIK, B.P. & YEH, F.C. 1983: Allozyme variability and evolution of lodgepole pine *(Pinus contorta* var. *latifolia)* and jack pine (*P. banksiana*) in Alberta. *Can. J. Genet. Cytol.* **25**: 57–64.

DOYLE, J.J. & DOYLE, J.L. 1990: Isolation of plant DNA from fresh tissue. *Focus* **12:** 145–151.

EL-KASSABY, Y.A. 1995: Evaluation of tree-improvement delivery system; factors affecting genetic potential. *Tree Physiol.* **15**: 545–550.

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.

FELSENSTEIN, J. 1993: PHYLIP (Phylogeny Inference Package) Ver. 3.5c. Department of Genetics. University of Washington, Seattle, USA. *Focus* **12**: 13–15.

FURNIER, G.R. & ADAMS, W.T. 1986: Geographic patterns of allozyme variation in Jeffery pine. *Amer. J. Bot.* **73**: 1009–1015.

GAST, S.J. & STOCK, M.W. 1994: Genetic diversity in overwintered and non-overwintered *Ips pini* (Say) (*Coleoptera, Scolytidae*) in Idaho. *Pan-Pac. Entomol.* **70**: 259–266.

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 Trees 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.

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

KOROL, L. & SCHILLER, G. 1996: Relations between native Israeli and Jordanian Aleppo pine (*Pinus halepensis* Mill.) based on allozyme analysis. *For. Genet.* **3:** 197 –202.

KOROL, L., SHKLAR, G. & SCHILLER, G. 2001: Diversity among circum-Mediterranean populations of Aleppo pine and differentiation from brutia pine in their isoenzymes: Additional results. *Silvae Genet.* **51**: 35–41.

KOROL, L., SHKLAR, G. & SCHILLER, G. 2002: Genetic variation within *Pinus halepensis* Mill. provenances growing in different microenvironments in Israel. *Isr. J. Plant Sci.* **50:** 135–143.

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

LEWONTIN, R.C. 1972: The apportionment of human diversity. *Evol. Biol.* **6:** 381–398.

LI, C.C. & HOROVITZ, D.G. 1953: Some methods of estimating the inbreeding coefficient. *Am. J. Hum. Genet.* **5**: 107–117.

LI, Y.C., FAHIMA, T. & PENG, J.H. 2000: Edaphic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. *Theor. Appl. Genet.* **101**: 1029–1038.

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.

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

MÜLLER-STARCK, G. 1987: Genetic differentiation among samples from provenances of *Pinus sylvestris. Silvae Genet.* **39**: 40–42.

MÜLLER-STARCK, G. 1995: Genetic variation under extreme environmental conditions. *In*: Population genetics and genetic conservation of forest trees. (eds. Baradat, Ph., Adams, W.T. and Müller-Starck, G.) SPB Acad. Pub. Amsterdam. pp. 201–210.

MULVEY, M., KELLER, G.P. & MEFFE, G.K. 1994: Single-locus and multiple-locus genotypes and life-history responses of *Gambusia holbrooki* reared at two temperatures. *Evolution* **48**: 1818–1819.

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 Genet.* **41**: 225 –233.

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

NEVO, E. 1997: Evolution in action across phylogeny caused by microclimatic stress at "Evolution Canyon". *Theor. Pop. Biol.* **52**: 231–243.

NEVO, E. 1998: Molecular evolution and ecological stress

at global, regional and local scales: The Israeli perspective. *J. Exper. Zool.* **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.

PRUS-GŁOWACKI, W., WOJNICKA-POLTORAK, A. & OLEKSYN, J. 1999: Industrial pollutants tend to increase genetic diversity: Evidence from field-grown European Scots pine populations. *Water, Air, Soil Poll.* **116**: 395–402.

SAFRIEL, N.U., VOLIS, S. & KARK, S. 1994: Core and peripheral populations and global climate change. *Isr. J. Plant Sci.* **42**: 331–345.

SAITOU, N. & NEI, M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

SAMBROOK, J., FRITSCH, E.F. & MANIATS, T. 1989: Molecular Cloning. A Laboratory Mmanual. 2nd edn. Cold Spring Harbor Laboratory Press, New York.

SCHILLER, G. 2000: Inter- and intraspecific 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., CONKLE, T. M. & GRUNWALD, C. 1986. Local differentiation among Mediterranean populations of Aleppo pine in their isoenzymes. *Silvae Genet.* **35**:11–19.

SCHILLER, G. & GRUNWALD, C. 1986: Xylem resin composition of *Pinus halepensis* Mill. in Israel. *Isr. J. Bot.* **35**: 23–33.

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. *For. Genet.* **6**: 257–276

SHOMER-ILAN, A. 1968: Drought resistance of pine seedlings. Ph. D. Thesis, The Hebrew University of Jerusalem, 85 pp (in Hebrew with English summary).

SLATKIN, M. 1985: Rare alleles as indicators of gene flow. *Evolution* **39:** 53–63.

SLATKIN, M. 1987: Gene flow and the geographic structure of natural populations. *Science* **236:** 787–792.

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

SWOFFORD, L.D. & SELANDER, B.R. 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.

VOLIS, S., MENDLINGER, S. & OLSVIG-WHITTAKER, L. 1998: Phenotypic variation and stress resistance in core and peripheral populations of *Hordeum spontaneum. Biodiver. Conserv.* **7**: 799–813.

WRIGHT, S. 1951: The genetical structure of populations. *Ann. Eugen.* **15:** 323–354.

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

YEH, F.C., YANG, R-C., BOYLE, T.B. J., YE, Z-H. & MAO, J.X. 1997: POPGEN, the Microsoft Windows-based user-friendly software for population genetic analysis of codominant and dominant markers and quantitative traits. University of Alberta, Canada. Department of Natural Resources.