conditions. Man has transferred forest tree seeds or seedlings around the Mediterranean, as has been established for *Pinus halepensis* and which is known to have happened in the case of *Pinus pinea*. Using isoenzyme analysis, it was found that *Pinus halepensis* growing in Umbria, Italy, have several alleles that are characteristic of *Pinus halepensis* growing in Israel and Jordan; historical evidence supports the hypothesis that seeds of the East Mediterranean group of *Pinus halepensis* were transferred to and planted in Umbria, Italy (SCHILLER *et al.*, 1985; SCHILLER and BRUNORI, 1992). *Pinus pinea* (stone pine; "*Pinus domestico*") is thought to originate in the Iberian peninsula, but because its seeds are edible this tree was transferred around the Mediterranean probably in Roman times (MIROV, 1967).

The highest values of observed and expected heterozygosity and total genetic diversity among *C. sempervirens* var. *horizontalis* recorded for the Turkish populations suggest that these populations might be relicts of the center of origin of this variety. On the other hand, the high diversity among the Israeli *Cupressus sempervirens*, indicates that these plantations might have come into being from several different origins.

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Genetic Diversity in *Pinus brutia* TEN.: Altitudinal Variation[1])

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Abstract

The aim of this study was to describe the genetic structure of *Pinus brutia* TEN. subsp. *brutia* (NAHAL, 1983) growing at different elevations in the Taurus Mountains in Turkey, and to use these data to define seed collection and transfer zones.

Isoenzyme analysis was performed on the maternal tissue of seeds to investigate the relationship between allele frequencies and altitude of populations occurring over a narrow geographic region in the vicinity of Antalya, in southern Turkey. Twenty-three loci encoding 14 enzyme systems were analyzed and 17 of these loci (69.6%), encoding 10 enzyme systems, were found to be polymorphic (69.6%). The mean genetic diversity within populations (H_s) was 0.263 and the mean total genetic diversity (H_t), 0.278, therefore, the proportion of total diversity among populations (G_{st}) was only 0.053; the mean degree of inbreeding within populations (F_{is}) was 0.167. Deficiency of heterozygotes was found in the Mnr-1, Mdh-4, 6Pgd-2 and Mpi loci. The results indicate that most of the genetic diversity in *P. brutia* is within populations. Significant relations were found between allele frequencies and altitude in Mdh-1, Mdh-4 and Skdh-1, Aco and Gdh enzyme systems. These results support earlier

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conclusions, based on several different methods of genetic research, concerning the existence of altitudinal clinal variation in *Pinus brutia*. subsp. *brutia* in the various traits analyzed. Hence, the importance of defining seed collection and transfer zones.

Key words: *Pinus brutia*, isozymes, altitudinal variation, cline.

FDC: 165.52; 113.2; 174.7 *Pinus brutia*; (560).

Introduction

Pinus brutia TEN. subsp. *brutia* (NAHAL, 1983) occurs in the eastern Mediterranean area, *i.e.*, mainly in the eastern part of the Aegean region, on Crete and Cyprus, and also sparsely along the shore of the Black Sea in Turkey, and in Syria, Lebanon and Iraq. It grows from sea level up to 1500 m in the Taurus Mountains (SELIK, 1958; CRITCHFIELD and LITTLE, 1966; MIROV, 1967), under several variations of the Mediterranean climate (EMBERGER et al., 1963), and on various bedrock formations and soils (ARBEZ, 1974). Recently, attention has been given to this sub-species, which is the most important forest tree in the region, providing both timber resources and amenity, especially in Turkey, Cyprus and Crete. This tree can be used for afforestation of degraded areas in the Mediterranean region and elsewhere, where there are homologues climates, because of its drought resistance (OPPENHEIMER, 1967). Thus, there is an urgent need to understand the nature and scale of diversity exhibited by the species, in order to aid the selection of suitable seed sources. Previous studies, based on the relatively sparse seed collections which covered the geographically wide range of this species (Anonymous, 1973; ARBEZ, 1974), used morphological, anatomical and biochemical traits to determine the extant of intra- and interpopulation genetic diversity. The results of such studies established the existence of altitudinal zonation within the wide geographic range of this sub-species, in allele frequencies (CONKLE et al., 1988), in cortex and needle resin composition (SCHILLER and GRUNWALD, 1987; SCHILLER and GENIZI, 1993), and in morphological and anatomical needle characters, resistance of seeds to water stress, and shoot morphology (CALAMASSI et al., 1980a and b, 1988; CALAMASS, 1982). A close examination of altitudinal variation within a narrow geographical region, based on seed and seedling characteristic was done by ISIK (1983, 1986). Later studies by ISIK (1993), ISIK and KAYA (1993), and YAHYAOGLU et al. (1993) yielded further evidence of higher intra- than interpopulation genetic variability.

The other 3 sub-species within the *Pinus brutia* complex, *i.e.,* subsp. *eldarica* (*Pinus eldarica* MEDW.); subsp. *pityusa* (*Pinus pityusa* STEVEN) and subsp. *stankewiczii* (*Pinus stankewiczii* [SUK.] FOM.) are rare pine species growing in few relatively small relict stands in the Crimea, on the coast of the Black sea and in Transcaucasia (MIROVE, 1967; NAHAL, 1983).

Some forest tree species are distributed over large geographical areas with considerable altitudinal variation, and the study of the geographical variation in their genetic characteristics, by means of isozyme gene markers, is one of the most useful aspects of forest research (CONKLE, 1992). The aim of our study, in which we used isozyme analysis, was 2-fold:

(1) To obtain more data on the geographical distribution of allele and genotype frequencies of isozymes from different populations of *Pinus brutia* subsp. *brutia* in more intensive sampling within a narrow, yet wide elevational range.

(2) To examine if any altitudinal variation appear in allozyme frequencies as it was the case in various morphological and biochemical traits.

Materials and Methods

Seed

Bulked seed material of *Pinus brutia* subsp. *brutia* from 9 natural stands (*Table 1*, *Figure 1*) distributed over a region covering 2° of longitude, 1.5° of latitude and 1000 m in altitude, in the vicinity of Antalya, Turkey, was obtained with the help of the Turkish Forestry Department. These stands are used by the Turkish Forest Service, as seed sources for the respective altitude zones in the close vicinity. The seeds were air dried and stored at 5 °C until they were used in the isoenzyme analysis.

Table 1. – Locations of *Pinus brutia* subsp. *brutia* populations analyzed.

Population (and closest town)		Abr.	Altitude (m)	Latitude N.	Longitude E.
Duzlercami	(Antalya)	Dzl.	275	36°59'	30°33'
Eskibag	(Gundogmus)	Esk.	1000	36°42'	32°10'
Guzelbag	(Gundogmus)	Gzl.	650	36°45'	31°58'
Karacay	(Kas)	Kry.	1050	36°24'	29°32'
Kargi	(Alanya)	Krg.	350	36°36'	31°57'
Kumluca	(Kumluca)	Kum.	250	36°26'	30°15'
Olimpos	(Antalya)	Oli.	320	36°35'	30°45'
Pinargozu	(Serik)	Pin.	500	37°16'	30°38'
Urlupelit	(Cevizli)	Url.	850	37°15'	31°45'

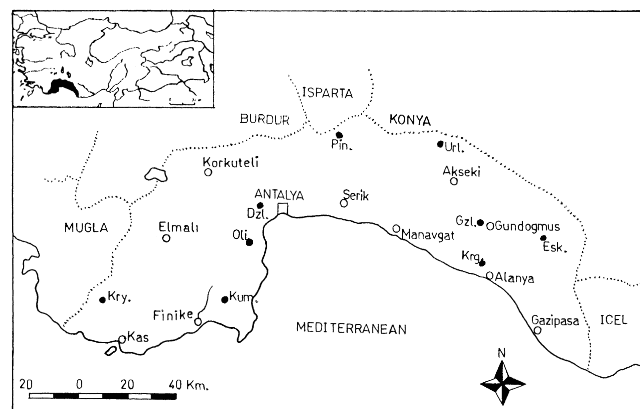

Figure 1. – Location of *Pinus brutia* TEN. populations included in the study. (Locations marked by black dots).

Electrophoretic Aalysis

For the analyses, seeds were germinated on moistened Whatman N3 filter paper, in Petri dishes at 20 °C.

Horizontal starch gel electrophoresis was used to obtain information on enzyme mobility variants in 23 loci encoding 14 enzyme systems. The megagametophyte tissue was homogenized in a grinding plate (KELLEY and ADAMS, 1977) with 130 µl of 0.2 M phosphate buffer pH 7.5, 0.1% Triton x-100, 1% BSA, and 0.1% β-mercaptoethanol for all enzyme systems. The liquid fraction from macerated tissue of haploid maternal seeds (megagametophytes) was analyzed simultaneously in 4 gel-buffer systems according to CONKLE et al. (1982).

System I, Gel buffer: 0.02M tris, 0.02M boric acid, 0.002M EDTA, pH 8.4. Electrode buffer: 0.2M tris, 0.2M boric acid, 0.002M EDTA, pH 8.4. Enzyme systems assayed: phos-

Table 2. – Allele frequencies of polymorphic loci in 9 natural populations of *Pinus brutia* TEN. in southern Turkey.

Population / Locus	Allele	Dzl.	Oli.	Krg.	Pin.	Kum.	Url.	Esk.	Gzl.	Kry.
ACO										
	1	0.137	0.156	0.192	0.169	0.092	0.269	0.215	0.195	0.133
	2	0.863	0.805	0.808	0.831	0.900	0.722	0.777	0.797	0.852
	3	0.000	0.039	0.000	0.000	0.008	0.009	0.008	0.008	0.016
ACP-2										
	1	0.669	0.547	0.515	0.585	0.485	0.414	0.508	0.406	0.688
	2	0.315	0.398	0.423	0.408	0.431	0.539	0.430	0.523	0.281
	3	0.000	0.039	0.000	0.000	0.015	0.000	0.000	0.000	0.000
	4	0.016	0.016	0.062	0.008	0.038	0.047	0.063	0.070	0.031
ADH-2										
	1	0.031	0.055	0.131	0.077	0.085	0.109	0.102	0.109	0.078
	2	0.969	0.945	0.869	0.923	0.915	0.891	0.898	0.891	0.922
GDH										
	1	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.000
	2	0.734	0.820	0.797	0.805	0.786	0.758	0.692	0.711	0.813
	3	0.202	0.180	0.203	0.195	0.214	0.242	0.308	0.242	0.188
GOT-1										
	1	0.815	0.938	0.908	0.516	0.669	0.672	0.700	0.688	0.805
	2	0.145	0.000	0.038	0.484	0.323	0.328	0.246	0.305	0.195
	3	0.040	0.063	0.054	0.000	0.000	0.000	0.054	0.008	0.000
	4	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000
GOT-2										
	1	1.000	0.875	0.985	0.875	0.908	0.914	0.915	0.930	0.883
	2	0.000	0.125	0.015	0.125	0.092	0.086	0.085	0.070	0.117
GOT-3										
	1	1.000	1.000	1.000	1.000	1.000	0.992	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
MDH-1										
	1	0.242	0.234	0.308	0.292	0.313	0.389	0.377	0.305	0.333
	2	0.758	0.766	0.692	0.708	0.688	0.611	0.623	0.695	0.667
MDH-4										
	1	0.871	0.875	0.854	0.915	0.828	0.836	0.731	0.672	0.727
	2	0.097	0.063	0.069	0.023	0.109	0.055	0.131	0.172	0.148
MNR-1										
	1	0.383	0.273	0.292	0.315	0.162	0.429	0.047	0.100	0.227
	2	0.533	0.523	0.592	0.500	0.615	0.571	0.414	0.800	0.570
	3	0.083	0.203	0.115	0.185	0.223	0.000	0.539	0.100	0.203
MNR-2										
	1	0.942	0.664	0.838	0.892	0.908	0.875	0.852	0.862	0.859
	2	0.058	0.336	0.162	0.108	0.092	0.125	0.148	0.138	0.141
MPI										
	1	0.810	0.609	0.731	0.016	0.269	0.078	0.625	0.531	0.453
	2	0.159	0.391	0.269	0.754	0.554	0.531	0.266	0.438	0.547
	3	0.000	0.000	0.000	0.230	0.177	0.367	0.000	0.000	0.000
	4	0.032	0.000	0.000	0.000	0.000	0.023	0.109	0.031	0.000
PGD-2										
	1	0.250	0.461	0.273	0.308	0.308	0.242	0.277	0.234	0.430
	2	0.750	0.539	0.727	0.692	0.692	0.758	0.723	0.766	0.570
PGD-3										
	1	0.677	0.719	0.669	0.754	0.692	0.711	0.656	0.578	0.656
	2	0.089	0.086	0.062	0.015	0.062	0.031	0.086	0.078	0.070
	3	0.234	0.195	0.269	0.231	0.246	0.258	0.258	0.344	0.273
PGI-2										
	1	0.578	0.578	0.654	0.685	0.646	0.211	0.777	0.664	0.617
	2	0.422	0.422	0.346	0.300	0.346	0.539	0.223	0.336	0.383
	3	0.000	0.000	0.000	0.015	0.008	0.250	0.000	0.000	0.000
SKDH-1										
	1	0.138	0.250	0.305	0.277	0.313	0.032	0.147	0.127	0.180
	2	0.457	0.306	0.141	0.223	0.281	0.347	0.328	0.405	0.336
	3	0.345	0.331	0.484	0.408	0.406	0.411	0.491	0.468	0.461
	4	0.000	0.081	0.063	0.054	0.000	0.073	0.000	0.000	0.000
	5	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.060	0.000	0.008	0.038	0.000	0.137	0.034	0.000	0.023
SKDH-2										
	1	0.795	0.820	0.797	0.962	0.932	0.891	0.906	0.813	0.883
	2	0.205	0.180	0.203	0.038	0.068	0.109	0.063	0.141	0.063
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.047	0.055

phoglucomutase (*Pgm*), menadione reductase *(Mnr)* and Alcohol dehydrogenase (*Adh*).

System II, Gel Buffer: 0.01M tris, 0.005M citric acid, pH 8.8. Electrode buffer: 0.05M NaOH, 0.3M boric acid pH 8.0. Enzyme systems assayed: glutamate-oxaloacetate transaminase (*Got*) and mannose phosphate isomerase (*Mpi*).

System III, Gel buffer: 0.002M citric acid, adjusted with morpholine [N-(3-aminopropyl)] to pH 6.1. Electrode buffer:

0.04M citric acid, adjusted with morpholine to pH 6.1. Enzyme systems assayed: aconitase (*Aco*), 6-phosphogluconate dehydrogenase (*6Pgd*), shikimate dehydrogenase (*Skdh*) and, leucine aminopeptidase (*Lap*).

System IV, Gel buffer: 0.002M citric acid, adjusted with morpholine to pH 8.3. Electrode buffer: 0.04M citric acid, adjusted with morpholine to pH 8.3. Enzyme systems assayed: malate dehydrogenase (*Mdh*), acid phosphatase (*Acp*), phosphoglucose isomerase (*Pgi*), glutamate dehydrogenase (*Gdh*), superoxide dismutase (*Sod).*

Gels were sliced and stained for each enzyme system according to CONKLE *et al*. (1982).

Statistics

Calculations of parameters of intra- and interpopulation genetic diversity (mean sample size per locus, mean number of alleles per locus, percentage of polymorphic loci, mean heterozygosity expected from HARDY-WEINBERG proportions, estimation of genetic differentiation and genetic distances, clustering and construction of dendrograms) were done by means of Bio-sys-1 a computer program for the analysis of allelic variation in genetics (SWOFFORD and SELANDER, 1981).

Use was also made of WRIGHT's (1951) equation to estimate the amount of gene flow among populations, *i.e.*, Nm, Nm = [(1-Fst)/4Fst]; where Fst is the variance of allelic frequencies among populations (MITTON, 1992).

Differences in rare or unique allele frequencies (F) between groups were estimated by FISHER's statistics (KRUTOVSKII and BERGMAN, 1995), *i.e.*, $F = [(\varphi_1 - \varphi_2)^2\ (N_1 * N_2)]/(N1 + N2)$, where $\varphi_1 = 2$ arcsin $(P_1)^{0.5}$ and) $\varphi_2 = 2$ arcsin $(P_2)^{0.5}$, P_1 and P_2 are the frequencies of rare and unique alleles, respectively, and N_1 and N_2 the total numbers of alleles in populations groups 1 and 2.

Results

Fourteen enzyme systems encoded by 23 loci were analyzed (*Table 2*). Six loci, *i.e.*, 26%, (Pgi, Pgm-1, Pgm-2, Idh-1, Sod-1 and Sod-2), encoding 4 systems, were monomorphic. Allele frequencies in the 17 polymorphic loci encoding 11 enzyme systems are shown in *table 2*. Seven loci (Pgi-2, Mnr-1, Mdh-4, Gdh, Aco, 6Pgd-3, Skdh-2) had 3 alleles; 6 loci (Mnr-2, Got-2, Got-3, Mdh-4, Adh-2, and 6Pgd-2) had 2 alleles; 3 loci (Got-1, Acp-2 and Mpi) had 4 alleles; and the locus Skdh-1 had 6 alleles.

Table 3. – Genetic variability among populations of *Pinus brutia* subsp. *brutia* near Antalia, Turkey.

Population	N	L	A	AP	P	Hobs	Hexp.	P(u)	Aep
Duzlercami	61.6	23	2.0± 0.2	3.13	65.2	.187± .041	.236± .045	–	1.43
Eskibag	64.4	23	2.1± 0.2	3.06	69.6	.217± .042	.271± .046	–	1.52
Guzelbag	64.0	23	2.1± 0.2	3.06	69.6	.251± .050	.279± .046	–	1.51
Karacay	64.0	23	2.0± 0.2	2.94	69.6	.217± .042	.276± .046	–	1.51
Kargi	64.7	23	2.0± 0.2	2.94	65.2	.214± .047	.255± .045	–	1.47
Kumluca	64.6	23	2.1± 0.2	3.06	69.6	.211± .043	.264± .048	.008	1.51
Olimpos	63.8	23	2.1± 0.2	3.00	69.6	.239± .046	.270± .049	.032	1.55
Pinargozu	64.6	23	2.1± 0.2	3.00	65.2	.189± .047	.251± .047		1.47
Urlupelit	63.4	23	2.2± 0.2	2.94	73.6	.246± .050	.282± .048	.008	1.56
Mean	63.9		2.1	3.01	69.6	.219	.265	.016	1.50
S.E.	0.3		0.02	0.02	0.02	.020	.005	.008	0.014

N = Mean sample size per locus; L = Number of loci analyzed; A = Mean number of alleles per locus;
AP = Mean number of polymorphic alleles per locus; P% = Percentage of polymorphic loci;
Hobs = Observed heterozygosity per population (direct count);
Hexp = Expected heterozygosity per population (unbiased estimate);
P(u) = Frequency of unique alleles; Aep = Effective alleles per locus.

Genetic variability within and among populations is presented in *table 3*. The overall mean percentage of polymorphic loci (P%) was 69.6% (range between 65.2% and 73.6%) and the overall mean expected heterozygosity (Hexp) was 0.265 (ranging between 0.236 and 0.282). Mean expected heterozygosity, in spite of the large S.E. of the means, increases very gradually but significantly with the raise in elevations (*Figure 2*). The overall mean number of alleles per locus (A) was 2.1 ± 0.02; the average number of alleles per polymorphic locus (AP) was 3.01 ± 0.023; and the mean effective number of alleles (Aep) was 1.50 ± 0.014. Comparison between the number of alleles per polymorphic locus (AP) and the effective number of alleles per locus (Aep) indicates that at least 2 common and several rare alleles are present across the populations. Of the 9 populations only 3 had private alleles, namely Skdh-1 (5th allele) in Olimpos, Got-1 (4th allele) in Kumluca and Got-3 (2nd allele) in the Urlupelit population (*Table 2* and *3*).

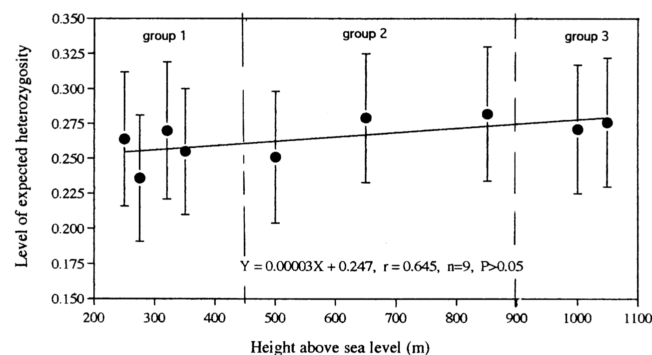

Figure 2. – Relations between mean expected heterozygosty of *Pinus brutia* TEN. populations and elevation in the Taurus Mountains near Antalya, Turkey. (Bars indicate s.d. of the mean).

Results achieved by the application of NEI's genetic diversity statistics (NEI, 1973) to our basic data are shown in *table 4*. The mean diversity within populations (Hs) was 0.263 (range from 0.002 to 0.660); the mean total diversity (Ht) was 0.278 (range from 0.002 to 0.682); the mean genetic diversity residing among populations (Gst) was, therefore very small, only 0.053 (range from 0.007 to 0.199). *Table 4* also presents the results of the F-statistics, *i.e.*, the genetic diversity coefficients, Fit, Fis and F_{st}, which were calculated according to WRIGHT (1978). The mean within locality inbreeding (Fis) over all loci among all nine populations was 0.147 (ranging from −0.151 to 0.751); suggesting a deficiency in heterozygotes in the loci of the following enzyme systems: Mdh-4 (0.751), Mnr_1 (0.655), Mpi (0.493) and 6Pgd-2 (0.221). The mean apparent value of F, *i.e.*, Fit, was 0.180 (range from −0.113 to 0.758). These results show that most of the genetic diversity in this species lies within populations and only a little between populations.

Levels of genetic differentiation between 3 elevation groups: 0 m to 450 m, 450 m to 950 m and 950 m to 1100 m above sea level are presented in *table 5*. Although inferences based on 2, 3 or 4 population per group are statistically weak, never the less heterozygosity within species (Hes) is lowest in the low elevation group and highest in the middle elevation group; and heterozygosity within population (Hep) is lowest at low altitude populations and highest at high altitude populations. Gst declines with raising altitude, *i.e.*, populations at similar altitude become more similar to each other as altitude increase. In other words, the higher Gst values exhibited by the low elevation group (*i.e.*, Kumulca and Duzlercami, Olimpos and Kargi, mean elevation of 299 m a.s.l.) of 0.085 indicate

Table 4. – Genetic diversity in *Pinus brutia* subsp. *brutia* (H and F statistic*).

Locus	Number of Alleles.	Ht	Hs	Gst	Fis	Fit
Aco	3	.302	.257	0.016	.166	.180
Acp_2	4	.539	.523	0.029	.104	.130
Adh_2	2	.158	.156	0.011	.137	.146
Gdh	3	.361	.357	0.011	.182	.191
Got_1	4	.391	.354	0.095	.065	.154
Got_2	2	.146	.143	0.025	.080	.103
Got_3	2	.002	.002	0.007	-.008	-.001
Mdh_1	2	.428	.423	0.011	.059	.069
Mdh_4	3	.326	.313	0.030	.751	.758
Mnr_1	3	.582	.537	0.077	.655	.681
Mnr_2	2	.248	.238	0.044	-.064	-.017
Mpi	4	.594	.475	0.199	.493	.594
$6Pgd_2$	4	.427	.415	0.028	.221	.242
$6Pdg_3$	3	.469	.462	0.009	-.015	-.006
Pgi_2	3	.502	.467	0.070	-.081	-.005
$Skdh_1$	4	.660	.682	0.032	-.083	-.049
$Skdh_2$	3	.235	.227	0.033	-.151	-.113
Mean	3	.278	.263	0.053	.147	.180
S.E.	0.019	.048	.045			

*) Ht = Total genetic diversity; Hs = Genetic diversity within populations; Gst = Dst/Ht, proportion of total diversity among populations; Fis = Degree of inbreeding within populations; Fit = Measure of the apperant value of (inbreeding coefficient), attributed to Fis and Fst.

Table 5. – Levels of allozyme variation within species, within populations and among populations in the 3 elevation groups.

A. Diversity within species	N	No. of Popula.	No. of Loci	Ps	As	Aes	Hes
All species	578	9	23	0.739	2.391	1.551	0.278
Group 1	256	4	23	0.696	2.261	1.511	0.263
Group 2	193	3	23	0.739	2.348	1.560	0.281
Group 3	129	2	23	0.696	2.130	1.534	0.278
B. Diversity within Populations				Pp	Ap	Aep	Hep
All species	578	9	23	0.696	2.097	1.505	0.263
Group 1	256	4	23	0.685	2.076	1.490	0.254
Group 2	193	3	23	0.710	2.130	1.519	0.269
Group 3	129	2	23	0.696	2.087	1.515	0.273
C. Diversity among Populations				Ht	Hs	Gst	Nm
All species	578	9	23	0.278	0.263	0.053	
Group 1	256	4	23	0.263	0.254	0.085	2.69
Group 2	193	3	23	0.281	0.269	0.036	6.69
Group 3	129	2	23	0.278	0.273	0.018	13.64

Group 1 is composed of the populations: Duzlercami, Kargi, Kumluca, Olimpos.
Group 2 is composed of the poulations: Guzelbag, Pinagozu, Urlupelit.
Group 3 is composed of the populations: Eskibag and Karacay.
Ps = Mean No. of polymorphic loci within species; As = Mean No. of alleles per locus within species; Aes = Mean No. off effective alleles per locus within species; Hes = Mean heterozygosity within species.
Pp = Mean No. of polymorphic loci within populations; Ap = Mean No. of alleles per locus within populations; Aep = Mean No. of effective alleles per locus within populations; Hep = Mean Heterozygostity within populations.
Ht = Total genetic diversity; Hs = Mean Genetic diversity within populations; Gst = Mean proportion of total diversity to differences between populations.
Nm = Gene flow between populations within a generation calculated according to WRIGHT (where N = effective population size; m rate of gene flow).

higher variation among these populations than among either the middle (*i.e.*, Pinargozu, Guzelbag, and urlupelit, mean elevation of 666 m a.s.l) of 0.036, or the higher elevation group (1025 m a.s.l.) of 0.018.

The genetic structure of the elevational groups (as defined in *table 5*) was also estimated in terms of the number of rare alleles in the populations. The mean frequencies of rare alleles were 0.027 in the lowest elevational group (299 m above sea level); 0.022 in the second group (666 m a.s.l.); 0.020 in the third group (1025 m a.s.l.). Therefore, values of FISHER's criterion are: 2.350 between the first and the second group; 0.173 between the second and the third group; and 3.204 between the first and the third group. Unique alleles (private alleles) were observed in three elevational groups; their mean frequencies were 0.008, 0.032 and 0.008 in the lowest, the second-lowest and the third group, respectively.

Relations between the mean allele frequency in the various loci analyzed and the altitude of the populations are presented in *table 6*. Significant regression equations between allele frequencies in a locus and altitude were found for the loci Mdh-1 (1st allele), Mdh-4 (1st and 3rd allele) and Skdh-1 (1st allele). Almost significant relations were found for Gdh and Aco also.

NEI's (1978) genetic identity calculations revealed a mean identity among the 9 analyzed populations of 0.981 ± 0.020 (range from 0.956 to 0.995). The population pairs that showed the lowest identity to one another were the Urlupelit and Eskibag (genetic identity of 0.956) and the Duzlercami and Pinargozu populations (genetic identity of 0.959). Analysis of single-locus genetic similarity (NEI, 1978) showed that the unbiased genetic identity coefficient of several loci, among populations, was less then 0.900. In the Urlupelit population, locus Pgi-2 had an average unbiased genetic identity with other populations of only 0.704 ± 0.07; in the Eskibag population, locus Mnr-1 had an average unbiased genetic identity of only 0.727 ± 0.104 with the other 8 populations. Low genetic identity coefficients of 0.895 between the Duzlercami population and the Urlupelit and Guzelbag populations, and of 0.873 between the Guzelbag and the Kas populations was found for locus Acp-2. The genetic identity coefficient among

Table 6. – Relations between population's mean allele frequency per locus and population's site altitud (n = 9).

Locus	allele	Regression equation	Correlation coefficient
Mdh_1	1	$Y = 0.237 + (1.3*10^{-4})X$	0.77**
Mdh_4	1	$Y = 0.910 - (1.7*10^{-4})X$	-0.65*
Mdh_4	3	$Y = 0.030 + (1.1*10^{-4})X$	0.81**
$Skdh_1$	1	$Y = 0.301 - (1.9*10^{-4})X$	-0.60*
$Skdh_1$	3	$Y = 0.360 + (1.1*10^{-4})X$	0.59
Aco	1	$Y = 0.126 + (0.8*10^{-4})X$	0.49
Gdh	3	$Y = 0.181 + (0.7*10^{-4})X$	0.53

*) significant at p = 0.05; **) significant at p = 0.01

several populations was low and varied for the Skdh-1 and Mpi loci. For Skdh-1 the coefficient was 0.855 ± 0.04 and for Mpi, 0.618 ± 0.212.

Cluster analysis resulting in a phylogenetic tree was based on EDWARDS' (1971, 1978) Euclidean distance procedure of rooting at the midpoint of longest path; the result after optimization is shown in *figure 3*. The 9 populations under consideration were clustered into 2 main branches, which then split into 5 subgroups mainly according to geographic proximity (see the map) and also the elevation of the sites. The Duzlercami, Olimpos and Kargi population of low elevation east of Antalya along the bay are clustered on the same brunch; the Eskibag and Guzelbag populations although differing in elevation are in close geographic proximity; the Pinargozu and Urlupelit populations too are in close geographic proximity and of middle elevation. The Kumluca and Karacay populations differ in their position (Groups c and e) are of close proximity to each other on the western side of the bay of Antalya, but of different elevation; this phenomenon can not be easily explained.

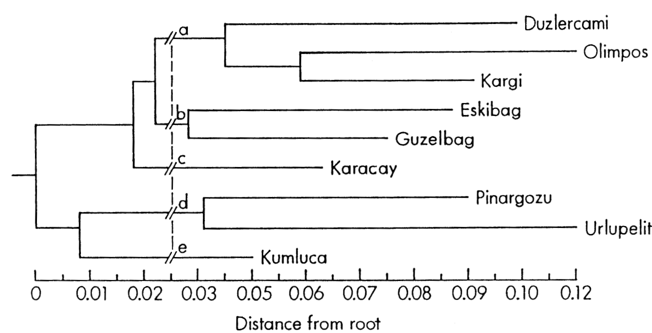

Figure 3. – Phylogenetic tree, based on "E" distance, showing the relations among 9 *Pinus brutia* populations near Antalya, Turkey.

Discussion

Earlier studies on variation in morphological, anatomical, protein, allozymes and resin characteristic have revealed the existence of considerable variation in the form and growth characteristics of this species (ARBEZ, 1974; CALAMASSI et al., 1988; ISIK, 1986; CONKLE *et al.*, 1988). Variation in most of these characteristics appeared to be related mostly to altitude and/or climatic factors.

Results presented in *tables 2* and *3* differ somewhat from those presented by CONKLE *et al.* (1988) who also analyzed 20 loci in 10 *Pinus brutia* populations from Turkey, Greece, Iraq and Cyprus (Duzlercami population was included in both CONKLE's and our studies). Percentage of polymorphic loci was only 43% in CONKLE's *et al.* (1988) compared with 69.6% in the present study, their mean expected heterozygosity was 0.118 ± 0.034 compared with 0.265 ± 0.005 in the present study. The mean number of alleles per locus found by CONKLE *et al.* (1988) was 1.53 ± 0.12 compared with 2.10 ± 0.02 in the present study. This differences may arise mainly from differences in population composition of these 2 studies.

Our results as well as the earlier ones (CONKLE *et al.*, 1988), suggest that the populations analyzed share a common gene pool, with minor geographic-physiographic differentiation. The phylogenetic tree (*Figure 3*) shows a geographic proximity and a clinal pattern, *i.e.*, low-altitude populations such as those of Duzlercami, Olimpos and Kargi form one distinct group, while 2 of the middle-elevation populations (namely Pinargozu and Urlupelit) form another distinct group. High-elevation populations (namely Karacay and Eskibag), although not clearly grouped together, are distinctly separated from both the low- and middle-altitude populations. Kumluca, which is the most westerly low-altitude population, remains as an outlier in the phylogenetic tree.

In a study of the genetic differentiation along an elevational transect of *Pinus ponderosa* (MITTON *et al.*, 1980), clinal differentiation was found at the Pgm locus. In the present study, significant positive or negative relations between allele frequencies and altitudes of populations were found for the Mdh and Skdh loci, and nearly significant ones for the Aco and Gdh loci (*Table 6*), which indicate clinal variation. There is somewhat higher intrapopulation genetic variability in the middle-elevation zone populations, as expressed in the heterozygosity level, than among either the lower- or the higher-elevation populations. The somewhat smaller intrapopulation genetic variability might be the result of selection pressure such as summer drought, and the strong fragmentation of the forest land and disgenic selective activity by humans among the easily accessible low-elevation populations during the last 2 millennia; while high-elevation populations are probably under the selection pressure of late and/or, early frosts during the growing season. Middle elevational zones in the Taurus Mountains, which offer an optimum growth habitat for *Pinus brutia,* were not easily accessible and free from exploitation in the past, which may contribute to the maintenance of higher heterozygosity within these populations.

According to WRIGHT's method (1951) there is an increase with altitude in the proportion of migrants per generation. Among the low-elevation groups, the proportions of migrants per generation are much smaller than among the middle- and high-elevation groups; migration among neighboring populations becomes less and less effective with decreasing elevation (*Table 5*). But, it should be remembered that migration is not the only evolutionary force that maintains similarity among populations. It is more likely that closer similarity among higher-elevation populations is not due to the contribution of migration, but rather the result of similar selection pressures imposed by the common influences of early and/or late frosts, at high elevations.

In conclusion, the results indicate that less stressful environments on the middle elevations of the Taurus Mountains are associated with higher total genetic diversity of the populations growing in this zone. The significant correlations between elevations and certain enzyme systems of populations found in the present study, and between elevation and various morphological characteristics found in earlier studies (ISIK, 1983, 1986; ISIK et al*.,* 1987; ISIK and KAYA, 1993) suggest the existence of a combined selection pressure exerted by human activity and climatic factors associated with sharp increase in altitude in the vicinity of Antalya, Turkey. These findings at the isoenzyme level support the earlier findings of ISIK (1983, 1986) that "*Pinus brutia* has locally adapted races, with predominantly clinal variation patterns" in various morphological, biochemical and isoenzyme characteristics.

Forestry practices such as selection of seed sources and determination of seed transfer zones should strongly emphasize the consideration of elevation gradients in this region.

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Genetic Analysis of Needle Proteins in Maritime Pine

1. Mapping Dominant and Codominant Protein Markers Assayed on Diploid Tissue, in a Haploid-Based Genetic Map

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Abstract

A genetic analysis of proteins was performed in a 3-generation inbred pedigree of maritime pine (*Pinus pinaster* AIT.). Proteins were extracted from needles (2n) and revealed by 2-dimensional gel electrophoresis. A total of 17 qualitative variants (presence/absence variations and position shifts) were observed and conformed to Mendelian inheritance patterns. These markers were localized in a previously reported genetic map based on RAPD markers assayed on megagametophytes (1n). To achieve this integration, evenly spaced RAPD markers were genotyped on diploid tissue. The internal amino acid sequences of 3 proteins were determined, and 2 of them

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