GENETIC DIFFERENTIATION OF NATURAL MEDITERRANEAN CYPRESS (*CUPRESSUS SEMPERVIRENS* L.) POPULATIONS IN GREECE

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ABSTRACT

The Mediterranean cypress has a disjunct geographic distribution in Greece. It mainly consists of small natural stands on Crete and on other Aegean islands, but it also has been introduced, mostly as the *pyramidalis* form, in many other localities. The nature and magnitude of genetic variation of natural and introduced stands are studied in the present paper. Twenty five populations representing different population histories, geographic localities and ecological site conditions were sampled. Zymograms of adult trees were obtained by means of starch gel electrophoresis of their seed perisperms. Large intrapopulational and moderate interpopulational variation was found. The greatest differences were observed among populations with different establishment histories. Natural populations showed much more variation within stands than introduced and ornamental ones. This indicates that introduced stands were founded by a few individuals. On the other hand, natural stands, which are the remnants of extensive forests of the past, somehow managed to avoid the bottleneck effect and to maintain high levels of heterozygosity and allelic diversity.

Keywords: *Cupressus sempervirens*, allozymes, differentiation, natural populations, artificial populations, Greece

INTRODUCTION

The Mediterranean cypress

The Mediterranean, or Italian, or common cypress (*Cupressus sempervirens* L.) grows over a wide natural range in diverse environments. Natural stands can be found in Northern Persia, Syria, Cyprus, Turkey and Greece (PAVARI, 1934). In Greece, it grows naturally on Crete and on some other Aegean islands. Its range is discontinuous, consisting mainly of populations growing over a wide latitudinal, longitudinal and altitudinal range, and in regions with diverse environment.

The Mediterranean cypress has two different varieties, both known since ancient times: The variety *horizontalis* has a broad crown and wide angles between branches and stem, and the variety *pyramidalis* has a conical form and small angles between branches and stem. The two varieties are interfertile and can give progenies with many types of crown structure (PANETSOS, 1967). The variety *horizontalis* is the natural one and can be found mainly in natural stands, but has also been introduced to many areas. The variety *pyramidalis* is the most widely planted of all the cypresses (JOHNSON, 1974). It has been cultivated since the ancient times for its columnar form (ZACHARIS, 1977). It should be found only in introduced populations, but it very often expands to form naturalized stands derived from a number of introduced individuals. It can also be found in mixed stands resulting from racial introgression from *pyramidalis* individuals planted near natural stands.

The cypress is part of the history, tradition and culture of the Mediterranean nations. The intense exploitation and the wars that have dominated this region have led to the degradation of the natural forests (ZACHARIS, 1977). The remaining stands grow in small populations under adverse climatic conditions, on eroded soils. They are presently endangered by athropogenic influences (wildfires, irrational harvesting and grazing) and disease, such as fungi of the genus *Seiridium* (WAGENER, 1939). Attacks of such pathogens have been already observed in some greek stands (XENOPOULOS, 1991). Yet nowadays, the importance of cypress seems to be greater than ever, since it is

well adapted to calcareous, clayish, dry and poor soils (XENOPOULOS *et al.*, 1990). Cypress trees can be used today as ornamental trees, boundary hedges, windbrakes, for soil and watershed protection. They also give good quality timber and essential oils (ANDREOLI and XENOPOULOS, 1990). Thus, cypress is one of the most important trees for multipurpose forestry in the Mediterranean region.

Objectives

The ability of the cypress to grow naturally on sites with diverse environmental conditions indicates a large genetic variation, as was confirmed fore some traits in field trials with Greek provenances (PANETSOS, 1967, 1992). Therefore, it is important to conserve the genetic variation of the natural stands, which are in danger of extinction, due to anthropogenic action and disease.

Before starting any breeding or conservation activity, it is important to know the amount and the nature of the existing genetic variation (PANETSOS, 1985). The aim of the present study is to use genetic structures of enzyme loci in order to supply the breeding and conservation research with knowledge about the amount and origin of the genetic variation in the greek stands. Comparison among the genetic structures of natural and introduced stands is of particular interest, in order to find any correlations between establishment history, adaptive characteristics and genetic structures.

MATERIAL AND METHODS

Studied populations

Seeds collected from single trees of 25 cypress stands (in this study referred to as populations) were sampled. The populations sampled represent stands with trees having various crown forms, origin and history (see map).The populations studied are classified into 4 groups according to their establishment history (Table 1):

Natural I: Natural populations of mostly high elevations, consisting exclusively of *horizontalis* individuals (9 populations).

Natural II: Natural populations of mostly lower elevations, that might have been in contact with planted *pyramidalis* individuals. The trees of these stands belong mostly to the *horizontalis* variety, but *pyramidalis* individuals or individuals with intermediate form can be found here and there (11 populations).

Introduced: Naturally expanded populations derived from introduced individuals. The tree crown form is intermediate or *pyramidalis* (3 populations).

Ornamental: Ornamental trees planted from nursery seeds. All trees belong to the variety *pyramidalis* (2 populations).

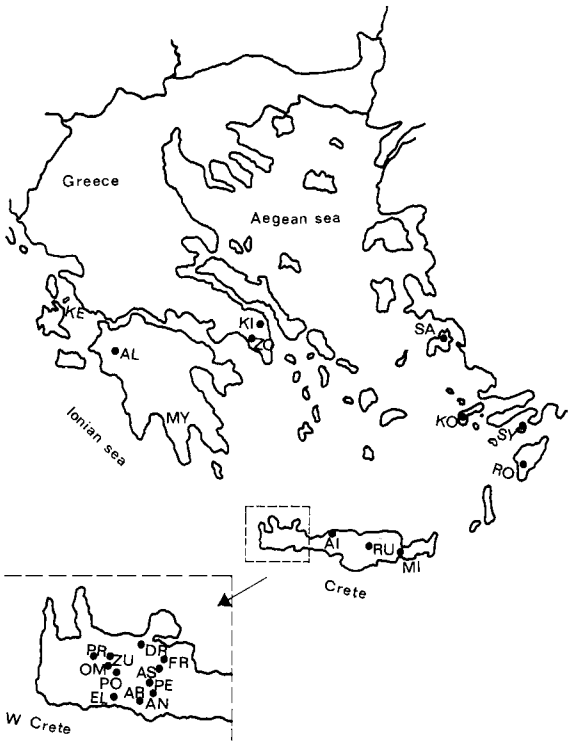

Figure 1 Distribution of investigated populations of the Mediterranean cypress in Greece

Eleven populations were sampled from western Crete, where most of the natural stands can be found. Two more were sampled from the eastern and one from the central part of the island. Four populations represent the Aegean islands Samos, Rhodos, Kos and Symi. One population was sampled from the Ionian island Kefallonia and two more from the Peloponnese. Two populations represent ornamental gardens in Athens. Finally, two populations come from a field trial of the Forest Research Institute of Athens with trees selected for their resistance against the fungus Seiridium cardinale. They originate from the islands Samos and Rodos.

Seed perisperma

Seeds of most coniferous species contain, besides the embryo tissue, the primary endosperm, which originates from the female megagametophyte. Coniferous

seed endosperm is haploid and represents genetically the female gamete that has contributed to the formation of the zygote. In the case of Mediterranean cypress seeds, non-embryonal tissue was found to be diploid. It seems, that the endosperm is connected with a diploid tissue, called seed perisperm (SINGH, 1978). The inability to isolate the endosperm from the perisperm in *Cupressus sempervirens* was first described by RADDI *et al.* (1990). Seed perisperm is generally described as a greatly compressed, thin, fleshy, cup-like tissue, which is the result of a strong compression of the nucellus (SINGH, 1978) and surrounds the female gametophyte (seed endosperm). Zymograms of seed perisperm represent the seed parent tree and not the female gamete. Electrophoretic studies of other species belonging to the *Cupressaceae* family, such as *Thuja plicata* D. DON, *T. orientalis* L., *T. occidentalis* L., *Chamaecyparis lawsoniana* (A. MURR.) PARL. and *Calocedrus decurrens* TORR. (FLORIN), used endosperm, as it is usual for most conifers (EL-KASSABY, 1981; HARRY, 1983; 1986; PERRY and KNOWLES, 1989; MILLAR and MARSHALL, 1991; XIE *et al.*, 1991a; 1991b; 1992). This is the case even for *C. macrocarpa* HARTW., which belongs to the genus *Cupressus* (KAFTON, 1976; CONKLE, 1986).

Whereas the existence of perisperm and the inability to isolate endosperms is a problem for genetic segregation analysis, it is also a big advantage for genotyping the seed parent, since we need to analyse only one seed per tree (PAPAGEORGIOU *et al.*, 1993). Moreover, there are many aspects of population genetics in which this tissue can play an important role.

Table 1 Studied populations

Population / Abbreviation		Area	Sample size	Altitude	Year	Crown form
Natural I						
Potamoi	PO	W. Crete	16	1750	1991	H
Aradena	AR	W. Crete	21	800	1991	H
Petres	PE	W. Crete	19	800	1991	H
Minos	MI	E. Crete	28	600 – 700	1992	H
Eligia	EL	W. Crete	10	500 – 700	1991	H
Ruwa	RU	E. Crete	27	1200	1992	H
Omalos	OM	W. Crete	18	1100	1990	H
Anopoli	AN	W. Crete	12	1500	1990	H
Askifu	AS	W. Crete	11	500 – 800	1990	H
Natural II						
Fress	FR	W. Crete	20	150	1990	H – P
Symi	SY	Aegean	20	100 – 250	1991	H
Ag. Ioannis	AI	C. Crete	21	100	1991	H – P
Rodos	RO	Aegean	21	150	1991	H – P
Rodos – R	ROR	trial	11	100	1992	H – P
Drakona	DR	W. Crete	20	150	1991	H
Samos	SA	Aegean	21	200	1991	H
Samos – R	SAR	trial	20	100	1992	H
Kos	KO	Aegean	21	250	1991	H
Zurva	ZU	W. Crete	20	450	1990	H
Prasses	PR	W. Crete	18	650	1990	H
Introduced						
Alepohori	AL	Peloponnese	28	100	1992	H – P
Mystras	MY	Peloponnese	29	350	1992	H – P
Kefallonia	KE	Ionian	30	50	1992	P
Ornamental						
Kifissia	KI	Athens	19	50	1991	P
Zografu	ZO	Athens	20	50	1992	P

H: Stands of *Cupressus sempervirens var. horizontalis*, P: Stands of *Cupressus sempervirens var. pyramidalis*, H – P: Stands having individuals of both varieties or with intermediate crown form

Electrophoresis

Horizontal starch gel electrophoresis was used to analyse the isozyme patterns of seven polymorphic enzyme systems in seed perisperms and needles. The following isozyme zones were assumed to be controlled by nine single loci (*Pgi-B, Pgm-A, Pgm-B, Gdh-A, 6Pgd-B, Mdh-B, Mdh-C, Lap-A, Ndh-A*); the genetic analysis described in the next section supports this assumption (PAPAGEORGIOU *et al.*, 1993). The electrophoretic procedures and the full names of the enzyme systems were given by BERGMANN (1974), CHELIAK and PITEL (1984) and WENDEL and WEEDEN (1989).

Genetic analysis

Regular meiotic segregation was tested previously for seven of nine loci used in this study. Embryos of single tree seed progenies derived from open pollination were used (GILLET and HATTEMER, 1989). The results confirmed the hypothesis of single loci with codominant alleles (PAPAGEORGIOU *et al.*, 1993). Two more loci (*Lap-A* and *Mdh-C*) were tested later using the same procedure. No significant deviation from the hypothesis of single loci with codominant mode of gene action was found in either case.

Measuring allele frequencies

LEWONTIN (1985) used the terms major and minor polymorphism, in order to classify isoenzyme polymorphisms qualitatively according to the kind of allelic frequency distribution within a population. Major polymorphism describes the case in which two or more alleles exist in a population with intermediate frequencies. Minor polymorphism represents the case in which one very common allele appears, followed by a number of rare alleles.

When an allele is not found in a population sample, it does not mean that this allele does not exist in the population; it is possible that it was not detected due to the small sample size. Thus we cannot say that a rare allele was present in one population but not in another. We also cannot directly compare the frequencies of rare alleles among different populations. We can only consider differences in the level of frequencies of an allele between two populations, if it has an intermediate or high frequency in at least one of the populations. The special case where one allele is rare or not found in one population but frequent or intermediate in another, indicates either reproductive isolation or a strong adaptational differentiation. This situation was observed during our study.

Measures of genetic variation

The following measures of intrapopulation genetic variation have been used for this study:

Gene pool diversity υ (GREGORIUS, 1978; 1987): Let a collection be characterized at locus l (l = 1,..,L) by the frequency vector $p_l = (p_{1l}, p_{2l}, ..., p_{nl})$ where $n \in N$ and for i = 1,...,n, $p_{il} \geq 0$ and $\sum_{i=1}^{n} p_{il} = 1$.

Denoting by $v_{(l)} = \left(\sum_{i=1}^{n} p_{il}^2\right)^{-1}$ the allelic diversity at the l-th locus, the gene pool diversity υ of the collection is given by the harmonic mean of the diversities of all loci and expresses the effective number of alleles of all loci.

Hypothetical gametic diversity υ_{gam} (GREGORIUS, 1978): For the same collection described above, the hypothetical gametic diversity υ_{gam} of the collection is defined as $v = \prod_{l=1}^{L} v(l)$ and is an expression of the effective number of multilocus gametic genotypes that can be produced in a population.

Average degree of heterozygosity H (GREGORIUS, 1978); the degree of heterozygosity is defined for an individual with respect to a specified number of gene loci, and is identical to the proportion of loci at which this individual is heterozygous. The average degree of heterozygosity refers to the distribution of this degree in a collection of individuals. Hence it can be proven that it equals the mean proportion of heterozygotes at the single loci.

Interpopulation variation was expressed by:

Genetic distance d_0 (GREGORIUS, 1986): Let one collection be characterized by the frequency vectors of the different genes (alleles) at L gene loci, that is, by L frequency vectors $p_l = (p_{1l}, p_{2l}, ..., p_{nl})$ (l = 1,..., L), where $n_l \in N$ is a number of alleles at locus l and $p_{kl} \geq 0$ and $\sum_{k=1}^{nl} p_{nl} = 1$ holds for all k = 1, ..., n_l. Let a second collection be characterized by the L frequency vectors $p'_l = (p_{1'l}, p_{2'l}, ..., p_{n'l})$ (l = 1, ..., L) at the same L loci and for the same numbering of alleles at each locus. The gene pool genetic distance d_0 between the two collections is defined as

$$d_0 = \frac{1}{L} \cdot \sum_{l=1}^{L} \left(\frac{1}{2} \cdot \sum_{k=1}^{n_l} |p_{k_l} - p^*_{k_l}| \right)$$

Population differentiation δ (GREGORIUS, 1986, 1987): Let a population be divided into demes (subpopulations, collections). The amount of genetic differentiation of one subpopulation to the remainder of the population is specified as "the proportion of genetic elements (alleles, genes at multiple loci, gametes, genotypes) by which a deme differs from the remainder of the population in type" (GREGORIUS, 1984). This proportion is defined as

$$D_j = d_0(p_j, \bar{p}_j) = \frac{1}{2} \sum_{k=1}^{n} |p_k - \bar{p}_k|$$

where p_j and p_j are the frequency distributions of the types in deme j and in the remainder of the population, respectively.

The subpopulation differentiation is then defined by

$\delta = \sum_j c_j \cdot D_j$ where the weights c_j express the proportion of genetic elements present in the j-th deme.

F_{ST} (WRIGHT, 1978):

$$F_{ST} = \left[\sum_{n=l}^{L} \sum_{i} \left(\sum_{j} c_j p_{in}^2(j) - p_{in}^2 \right) \right] \left[\sum_{n=l}^{L} \sum_{i} p_{in}(1 - p_{in}) \right]$$

Likelihood ratio (G) and χ^2 tests (SOKAL and ROHLF, 1981) were used to examine homogeneity of the absolute allelic frequencies.

Table 2a Allele frequencies of *Pgi-B*, *Pgm-A* and *Pgm-B* of Mediterranean cypress populations

Population	*Pgi-B*		*Pgm-A*		*Pgm-B*		
	1	2	1	2	1	2	3
Potamoi	0.750	0.250	0.375	0.625	0.000	0.406	0.594
Aradena	0.833	0.167	0.500	0.500	0.250	0.450	0.300
Petres	0.763	0.237	0.579	0.421	0.000	0.474	0.526
Minos	0.857	0.143	0.600	0.400	0.062	0.375	0.563
Eligia	0.750	0.250	0.450	0.550	0.000	0.400	0.600
Ruwa	0.722	0.278	0.704	0.296	0.037	0.481	0.481
Omalos	0.889	0.111	0.556	0.444	0.000	0.417	0.583
Anopoli	0.417	0.583	0.455	0.545	0.000	0.545	0.455
Askifu	0.818	0.182	0.591	0.409	0.000	0.682	0.318
Fress	0.675	0.325	0.425	0.575	0.050	0.475	0.475
Symi	0.737	0.263	0.711	0.289	0.026	0.342	0.632
Ag. Ionnis	0.643	0.357	0.262	0.738	0.050	0.475	0.475
Rodos	0.548	0.452	0.595	0.405	0.000	0.238	0.762
Rodos – R	0.773	0.227	0.545	0.455	0.000	0.455	0.545
Drakona	0.700	0.300	0.583	0.417	0.028	0.500	0.472
Samos	0.762	0.238	0.700	0.300	0.025	0.375	0.600
Samos – R	0.750	0.250	0.675	0.325	0.000	0.525	0.475
Kos	0.625	0.375	0.575	0.425	0.000	0.425	0.575
Zurva	0.775	0.225	0.450	0.550	0.025	0.450	0.525
Prasses	0.556	0.444	0.667	0.333	0.000	0.417	0.583
Alepohori	0.804	0.196	0.893	0.107	0.000	0.780	0.220
Mystras	0.603	0.397	0.983	0.017	0.000	0.346	0.654
Kefallonia	0.259	0.741	0.483	0.517	0.000	0.750	0.250
Kifissia	0.389	0.611	0.778	0.222	0.000	0.667	0.333
Zografu	0.650	0.350	0.833	0.167	0.000	0.583	0.417

Table 2b Allelic frequencies of *Ndh-A* and *6-Pgdh-B* of Mediterranean cypress populations

Population	*Ndh-A*			*6Pgdh-B*		
	1	2	3	1	2	3
Potamoi	0.250	0.531	0.219	0.344	0.594	0.062
Aradena	0.357	0.548	0.095	0.333	0.548	0.105
Petres	0.289	0.474	0.237	0.395	0.500	0.105
Minos	0.250	0.536	0.214	0.333	0.537	0.130
Eligia	0.150	0.650	0.200	0.500	0.450	0.050
Ruwa	0.130	0.741	0.130	0.407	0.444	0.148
Omalos	0.278	0.389	0.333	0.361	0.583	0.056
Anopoli	0.292	0.625	0.083	0.500	0.500	0.000
Askifu	0.409	0.364	0.227	0.227	0.591	0.182
Fress	0.125	0.750	0.125	0.350	0.325	0.325
Symi	0.079	0.737	0.184	0.500	0.342	0.158
Ag. Ionnis	0.333	0.524	0.143	0.214	0.476	0.310
Rodos	0.175	0.550	0.275	0.429	0.429	0.143
Rodos – R	0.136	0.818	0.045	0.591	0.227	0.182
Drakona	0.175	0.650	0.175	0.475	0.400	0.125
Samos	0.310	0.548	0.143	0.262	0.476	0.262
Samos – R	0.275	0.675	0.050	0.450	0.425	0.125
Kos	0.214	0.500	0.286	0.500	0.175	0.325
Zurva	0.250	0.625	0.125	0.400	0.500	0.100
Prasses	0.222	0.500	0.278	0.417	0.361	0.222
Alepohori	0.000	1.000	0.000	0.214	0.125	0.661
Mystras	0.000	0.966	0.034	0.241	0.310	0.448
Kefallonia	0.190	0.810	0.000	0.448	0.034	0.517
Kifissia	0.000	0.947	0.053	0.059	0.500	0.441
Zografu	0.050	0.900	0.050	0.361	0.139	0.500

RESULTS

Allele frequencies

Allele frequencies for all populations and gene loci are presented for each locus separately in table 2. Allele frequencies observed at all loci in all populations were tested for homogeneity (G and χ^2 tests). Highly significant ($p < 0.001$) differences were observed at *Gdh-A, Lap-A, Ndh-A, Pgi- B, 6Pgdh-B, Pgm-A* and *Pgm-B*. Allele frequencies at *Mdh-B* and *Mdh-C* did not show significant differences, because they were almost monomorphic.

Different types of polymorphism appeared among the populations at the same locus. The largest differences were found at the locus *Lap-A*, where we had in many cases a major polymorphism with two very frequent alleles (A_4 and A_5, the other alleles had moderate or high frequencies. The extreme case was population Askifu (AS), in which all six alleles had almost the same frequency. However, the sample size of this population was small. Changes from minor to major polymorphism could also be seen in *Pgm-A, Ndh-A* and *6Pgdh-B*.

Variation within populations

All measures used to express variation within populations (table 3) showed the same clear tendency: Each of the populations belonging to the groups Natural I and II had more variation than any of the populations belonging to the groups Introduced and Ornamental.

The maximum values for gene pool diversity υ and hypothetical gametic diversity υ_{gam} were observed in Western Crete (PE and AS respectively). The minimum values of these two measures were observed in one and the same population: Alepohori (AL – Peloponnese), an introduced stand with mixed crown form. Introduced and Ornamental stands showed much less variation within populations; the maximum values of these groups (KE) were smaller than the minimum values of the groups Natural I and II (SY). In order to

better observe the differences, we pooled all data belonging to the same group. The measures of variation within populations can be seen in table 4a.

The average degree of heterozygosity for all loci showed the same tendency. The higher value of the groups Ornamental and Introduced (H = 0.333 for KE) was exactly equal to the lowest value of the groups Natural I and II (ROR). The highest heterozygosity was observed in Western Crete (H = 0.489 for EL) and the lowest in Kifissia (H = 0.206), an ornamental garden in Athens. This tendency, namely higher values of H, υ and υ_{gam}, in natural populations is expressed in table 4a as well.

The mean observed heterozygosity of all populations examined was 0.364.

Variation among populations

Genetic distances d_0 for all populations examined were estimated, and a dendrogramme (figure 2) was constructed using these values based on the UPGMA method (SNEATH and SOKAL, 1973). Two main groups appeared at the level of d_0 = 0.20: The populations belonging to the classes Natural I and II form one group, while Introduced and Ornamental populations form the other. The populations of the second group

Table 2c Allele frequencies of *Mdh-B* and *Gdh-A* of Mediterranean cypress populations

Population	*Mdh-B*			*Gdh-A*			
	1	2	3	1	2	3	4
Potamoi	0.000	0.000	1.000	0.062	0.031	0.031	0.875
Aradena	0.048	0.000	0.952	0.000	0.075	0.300	0.625
Petres	0.000	0.000	1.000	0.158	0.132	0.095	0.632
Minos	0.000	0.000	1.000	0.000	0.321	0.143	0.536
Eligia	0.000	0.000	1.000	0.000	0.050	0.050	0.900
Ruwa	0.000	0.019	0.981	0.000	0.220	0.180	0.600
Omalos	0.028	0.000	0.972	0.083	0.278	0.056	0.583
Anopoli	0.000	0.000	1.000	0.000	0.062	0.125	0.813
Askifu	0.000	0.000	1.000	0.222	0.111	0.111	0.556
Fress	0.000	0.000	1.000	0.000	0.000	0.083	0.917
Symi	0.000	0.000	1.000	0.000	0.132	0.053	0.816
Ag. Ionnis	0.000	0.024	0.976	0.071	0.024	0.095	0.810
Rodos	0.000	0.000	1.000	0.143	0.143	0.095	0.619
Rodos – R	0.045	0.000	0.955	0.111	0.222	0.000	0.667
Drakona	0.000	0.000	1.000	0.029	0.059	0.088	0.824
Samos	0.000	0.000	1.000	0.214	0.167	0.071	0.548
Samos – R	0.000	0.000	1.000	0.100	0.225	0.000	0.675
Kos	0.025	0.000	0.975	0.139	0.056	0.111	0.694
Zurva	0.025	0.000	0.975	0.000	0.233	0.067	0.700
Prasses	0.028	0.028	0.944	0.000	0.139	0.056	0.806
Alepohori	0.000	0.000	1.000	0.000	0.232	0.018	0.750
Mystras	0.000	0.000	1.000	0.017	0.121	0.000	0.862
Kefallonia	0.000	0.000	1.000	0.018	0.018	0.056	0.806
Kifissia	0.000	0.000	1.000	0.026	0.053	0.053	0.868
Zografu	0.000	0.000	1.000	0.000	0.029	0.000	0.971

have large genetic distances from each other, especially Kefallonia (KE). The first group of the Natural populations is divided into three major subgroups at the level of d_0 = 0.15: The first includes the populations AR and AS (Western Crete, Natural I), the second subgroup Cretan populations belonging mainly to the group Natural I (except AI, FR, ZU, which belong to the group Natural II) and the third populations of Crete and Aegean islands, all belonging to Natural II except RU. These results show no geographic tendency. The factor that seems to most influence the classification observed in the dendrogram is the establishment history of the stands.

The genetic distances d_0 among the pooled populations (table 4b) showed the same grouping. Natural I has a small genetic distance (0.068) to Natural II.

Introduced has a small distance (0.072) to Ornamental as well. These two groups are separated by large distances.

Subpopulation differentiation δ was 0.127 among all populations. This measure equated 0.099 among all Natural populations as well as among all Cretan populations and 0.095 among the Aegean island populations. F_{ST} equaled 0.064 among all populations studied.

DISCUSSION

Large differentiation was observed for the Mediterranean cypress generally. Clear tendencies related to the origin of the stands were observed for the allele frequencies at four of the nine loci. At *Gdh-A,* allele A_4 was the most frequent in all populations, although the three other alleles were very rare only in the Introduced and Ornamental ones. The same tendency was observed for allele A_2 in *Ndh-A.* The other two alleles (A_1 and A_3) became rare or were not detected at all in the Introduced and Ornamental group. The most extreme case was Alepohori (AL), where no allele other than A_2 was detected. In *Pgi-B,* allele B_1 was more frequent in the Natural stands than in the Ornamental and Introduced ones; the opposite happened with allele B_2. A similar tendency was shown in *Pgm-A:* Alleles A_1 and A_2 had almost equal frequencies, building a major polymorphism in the Natural stands I and II. Allele A_1 became more frequent in the Introduced and Ornamental stands, becoming a typical minor polymorphism (MY).

with allele B_2. A similar tendency was shown in *Pgm-A:* Alleles A_1 and A_2 had almost equal frequencies, building a major polymorphism in the Natural stands I and II. Allele A_1 became more frequent in the Introduced and Ornamental stands, becoming a typical minor polymorphism (MY).

Table 2d Allelic frequencies of *Lap-A* and *Mdh-C* of Mediterranean cypress populations

Population	*Lap-A*						*Mdh-C*	
	1	2	3	4	5	6	1	2
Potamoi	0.000	0.219	0.312	0.312	0.156	0.000	1.000	0.000
Aradena	0.095	0.048	0.119	0.429	0.310	0.000	1.000	0.000
Petres	0.026	0.132	0.263	0.342	0.184	0.053	1.000	0.000
Minos	0.020	0.040	0.240	0.460	0.240	0.000	1.000	0.000
Eligia	0.150	0.050	0.150	0.500	0.100	0.050	1.000	0.000
Ruwa	0.037	0.130	0.056	0.593	0.185	0.000	1.000	0.000
Omalos	0.000	0.083	0.194	0.417	0.306	0.000	1.000	0.000
Anopoli	0.000	0.136	0.318	0.409	0.136	0.000	1.000	0.000
Askifu	0.136	0.136	0.136	0.227	0.182	0.182	1.000	0.000
Fress	0.000	0.075	0.200	0.550	0.175	0.000	1.000	0.000
Symi	0.053	0.000	0.132	0.605	0.211	0.000	1.000	0.000
Ag. Ionnis	0.000	0.175	0.150	0.350	0.325	0.000	1.000	0.000
Rodos	0.000	0.095	0.048	0.548	0.310	0.000	1.000	0.000
Rodos – R	0.000	0.000	0.187	0.438	0.375	0.000	1.000	0.000
Drakona	0.028	0.028	0.083	0.722	0.139	0.000	1.000	0.000
Samos	0.024	0.024	0.071	0.619	0.262	0.000	1.000	0.000
Samos – R	0.025	0.000	0.100	0.675	0.200	0.000	1.000	0.000
Kos	0.025	0.075	0.075	0.425	0.400	0.000	1.000	0.000
Zurva	0.125	0.125	0.250	0.350	0.150	0.000	0.950	0.005
Prasses	0.000	0.278	0.111	0.306	0.306	0.000	1.000	0.000
Alepohori	0.000	0.000	0.036	0.304	0.661	0.000	1.000	0.000
Mystras	0.000	0.069	0.069	0.310	0.552	0.000	1.000	0.000
Kefallonia	0.019	0.135	0.192	0.365	0.288	0.000	1.000	0.000
Kifissia	0.000	0.000	0.029	0.365	0.324	0.000	1.000	0.000
Zografu	0.000	0.150	0.425	0.100	0.100	0.000	1.000	0.000

In all tendencies referred to so far, the population of Kefallonia (KE) has almost always been an exception. This population belongs to the group of the Introduced populations, but has more and different alleles than the Introduced stands of Peloponnese (MY, AL). A reason for this observation is most probably the history of this island: Kefallonia was part of Italy for centuries. Its stands were protected when the neighboring Peloponnese was totally burned during the Greek revolution (1821 – 1830). Considering this, there has been no founder effect, and the Kefallonian population has probably never gone through a genetic bottleneck.

The results from the allele frequencies and the trends observed point out the effect of the population origin and history on the genetic structures.

The variation within populations was also strongly related to the classification according to establishment history. Natural populations I and II had larger variation than Introduced and Ornamental stands. This was obvious in all measures used. The difference between the Natural and Introduced or Ornamental stands can also been shown by the measures describing the within-group variation of the data pooled according to groups.

These results can be explained by the effective founder population size of the stands. The similarity of the genetic structures between the Introduced stands of Peloponese and the ornamental gardens of Athens indicates their similar establishment history and thus their origin from a small number of individuals. Introduced stands are considered natural by the local people, but the form of the trees (*pyramidalis* or mixed) and the results of this study show that these stands were derived from a few introduced individuals, which had been imported in the past and had remained after the total destruction of the peninsula during the Greek revolution.

The differentiation found among all populations was less than that within populations. In comparison to other species (see below), we can say that differentiation among populations is moderate. The grouping observed in the dendrogram (fig. 1) is due to the different origins of the populations.

Stands growing not far from each other showed different genetic structures and had large genetic distances. A special note should be made of the population of Aradena (AR), which grows not far from other populations (approximately 2 km). Yet, in Aradena, two alleles ($Pgm\text{-}B_1$ and $Gdh\text{-}A_3$) which were rare or not found in neighboring populations were detected with intermediate frequencies. This may be explainable by a large adaptational differentiation between this population and the others. The presence of many of the same rare alleles in almost all populations supports the hypothesis that strong gene flow had probably protected the small populations from genetic drift.

Table 3 Variation within populations

Population	H	υ	υ_{gam}
Potamoi	0.410	1.613	151.288
Aradena	0.433	1.751	311.693
Petres	0.386	1.757	372.285
Minos	0.374	1.702	248.542
Eligia	0.489	1.575	109.283
Ruwa	0.387	1.659	158.863
Anopoli	0.352	1.639	152.660
Askifu	0.481	1.756	480.926
Omalos	0.451	1.691	236.279
Symi	0.340	1.549	79.580
Fress	0.333	1.585	120.787
Ag. Ioannis	0.427	1.702	247.193
Rodos	0.383	1.699	214.861
Rodos – R	0.333	1.617	119.341
Samos	0.394	1.687	205.506
Samos – R	0.411	1.595	102.246
Drakona	0.363	1.581	96.202
Kos	0.426	1.752	284.883
Zurva	0.378	1.742	285.950
Prasses	0.407	1.749	313.858
Alepohori	0.218	1.325	16.901
Mystras	0.282	1.386	35.147
Kefallonia	0.333	1.498	70.363
Kifissia	0.206	1.413	32.605
Zografu	0.301	1.438	50.497

Table 4a Variation within groups

Group	H	υ	υ_{gam}
Natural I	0.414	1.746	308.168
Natural II	0.385	1.714	230.671
Introduced	0.277	1.483	58.717
Ornamental	0.258	1.472	62.733

All historical facts report that the present natural populations are the remnants of an extensive cypress forest of the past. After the forest destruction, the small populations survived, but they did not go through genetic bottlenecks that could have been caused by even smaller numbers of individuals. The maintenance of variation has probably been due to gene flow, which kept the remaining populations in contact. On the contrary, founder effects could not be avoided by the introduced populations of Peloponnese,

as shown by the small intrapopulational variation of these stands. Thus, we observe two different reactions to rapid reduction of population size: Natural stands avoided genetic bottlenecks, while introduced stands suffered loss of variation.

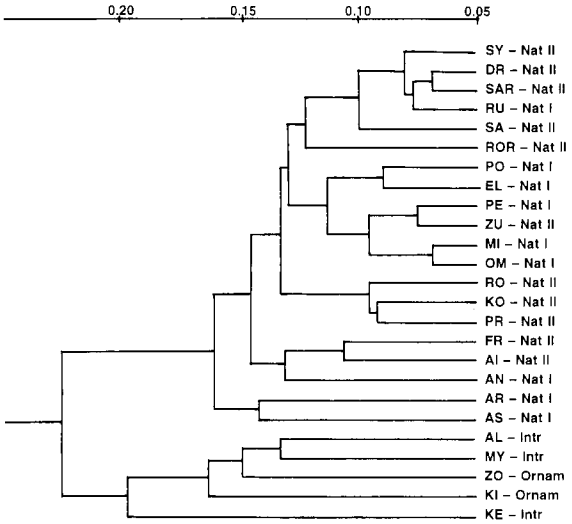

Figure 2 Dendrogramme based on genetic distances among populations. **NatI** – populations belonging to the group Natural I; **NatII** – populations belonging to the group Natural II; **Intr** – populations belonging to the group Introduced; **Ornam** – populations belonging to the group Ornamental.

The high heterozygosity of natural stands is probably explained by a very strong viability selection, due to adverse environmental conditions and by the temporal variation of climatic conditions. Besides the small genetic base, another explanation of the lack of variation in the introduced stands could be the strong artificial selection for tree form performed by humans in the introduced variety *pyramidalis* for thousands of years.

Many authors have tried to relate genetic variation and its origin to the geographic distribution of the species examined (CONKLE, 1992, HAMRICK *et al.*, 1992, MÜLLER-STARCK *et al.*, 1992). The results of isoenzyme variation studies in many species were grouped and conclusions were drawn. The geographic range was used as predictor of allozyme variation levels in perennial woody plants (HAMRICK *et al.*, 1992) Species with small, disjunct geographical ranges were reported to have large interpopulational and small intrapopulational variation (CONKLE, 1992, HAMRICK *et al.*, 1992, MÜLLER-STARCK *et al.*, 1992). However, some exceptions to this rule were reported as well (CONKLE, 1986, 1992, MÜLLER-STARCK *et al.*, 1992). One of these exceptional cases is most probably the Mediterranean cypress. Mean observed heterozygosity (H = 0.364) was higher than for many other, not only disjunct but also widespread tree species. The variation among cypress populations (δ = 0.127, F_{ST} = 0.064) was moderate in comparison to disjunct species but higher than widespread species.

Not only the Mediterranean cypress is the exception to this classification: *Pinus longaeva* D. K. BAILEY (HIEBERT and HAMRICK, 1983), *Cupressus macrocarpa* HARTW. (CONKLE, 1986), *Pinus leucodermis* ANT. (MORGANTE und VENDRAMIN, 1990), *Taxus baccata* L. (THOMA, 1992), *Nothofagus truncata* (COLENSO) COCKAYNE (HAASE, 1992) and other tree species with disjunct geographic ranges do not follow the observations of the grouping and show large intra- or small interpopulational genetic variation.

Considering the results mentioned above, it is doubtful that all disjunct or endemic species show a certain trend and have similar genetic structure. Factors other than geographic dispersal may also play a role. In the case of *Cupressus sempervirens* in Greece, establishment history and origin of populations are very important.

Natural cypress stands, most of which grow under adverse environmental conditions, contain considerable genetic variation and are valuable for the conservation of the species. No other conservation strategy seems to be needed than to put an end to irrational management of the ecosystems studied and to anthropogenic destruction.

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