CANARY ISLANDS PINE (PINUS CANARIENSIS CHR. SM. EX DC.) 1. DIFFERENTIATION AMONG NATIVE POPULATIONS IN THEIR ISOENZYMES*

G. Schiller¹**, L. Korol¹, E. D. Ungar¹, A. Zehavi³, S. L. Gil² & M. J. Climent²

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 ¹⁾ Department of Agronomy and Natural Resources, The Agricultural Research Organization, The Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel. Fax:-972-3-9669642; e-mail: vcgabi@agri.gov.il
 ²⁾ Department of Anatomy, Physiology and Forest Genetics, The Polytechnic University of Madrid, Madrid, Spain.
 ³⁾ R&D Unit of the Forest Department, Land Development Authority (KKL), Eshtaol, Israel.
 ** To whom correspondence should be addressed

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ABSTRACT

The aim of this study was to evaluate the level and structure of the genetic diversity of *P. canariensis*. The overall genetic diversity in 21 natural populations and one artificial population at 32 loci showed that: (a) the overall mean number of alleles per locus (A) was 1.76, with a range from 1.38 to 2.00; (b) the overall mean number of alleles per polymorphic locus (A_p) was 2.45, with a range from 2.22 to 2.80; (c) the overall mean proportion of polymorphic loci (P) at the 0.99 criterion was 0.511 with a range from 0.281 to 0.656; (d) the overall mean observed heterozygosity was 0.117 with a range from 0.070 to 0.169; (e) the overall mean expected heterozygosity was 0.125 with a rage from 0.079 to 0.149. Differences among populations and islands, in the numbers and average frequencies of private alleles, were detected. Populations growing at between 500 and 1000 m a.s.l. hade more private alleles than those growing at higher elevations. Parameters of genetic diversity at species level showed that: (a) total gene differentiation (H_i) was 0.134; (b) within-population gene differentiation (H_s) was 0.122; (c) G_{sr} , the proportion of total diversity among populations was 0.091, which means that most of the diversity lies within populations. Chi-squared analysis for allele frequency differences among populations showed significance for 19 of the 28 polymorphic loci at the level of p < 0.05. Consideration of the genetic structure parameters at the level of islands and ecological and/or altitudinal zonation revealed that each island is unique in its genetic structure, because of differences among islands in the numbers of monomorphic loci; significant influences of geoclimatic parameters on allele frequencies were revealed.

Key words: population genetics, genetic diversity, genetic structure, isoenzymes, Pinus canariensis

INTRODUCTION

The Canary Islands are known to be a refuge for a large number of species from the Macaronesian and the Mediterranean-Tertiary flora among others (BRAMWELL 1976). Pinus canariensis Chr. Sm. ex DC. (Canary Island pine) is a relict occurrence of a species which, in the lower Cretaceous and Tertiary, extended from the Himalayas to the Atlantic, in lands bordering the northern shores of the Tethys Sea (KASAPLIGIL 1978; MERCER 1980); it is endemic to five of the seven Canary Islands (Tenerife, La Palma, Gran Canaria, El Hierro and Gomera). These islands lie between latitudes 27° 30' and 28° 30' N and longitudes 15° 00' and 18° 00' W, and are 270-580 km west of Cape Juby, Morocco. The islands are of volcanic origin of the pre-Cretaceous era. Canary Island Pine forests grow from about 500 to 2300 m a.s.l., over a wide range of habitats, which differ in slope inclination and aspect, bedrock formations and soil properties. Climatic conditions on this mountainous islands vary considerably over short distances, so that *P. canariensis* grows there under widely differing ecological conditions (BLANCO-ANDRAY *et al.* 1989; BRAMWELL 1976; CLIMENT-MALDONADO *et al.* 1996; CRITCHFIELD & LITTLE 1966; FERNANDOPULLE 1976; MERCER 1980; MIROV 1967; SCHMINCKE 1976).

Within his attempt to clarify the phylogenetic relations among Mediterranean pine species, KLAUS (1989) concluded that phenotypes of *P. canariensis* contain features of almost all Mediterranean shore pines. The relations suggested by KLAUS (1989) were strongly supported by analysis of chloroplast DNA restriction site mutation (KRUPKIN *et al.* 1996), which clustered *P. canariensis, P. pinea* and *P. brutia* in the same clade. All this suggests "that *P. canariensis* is an old relict from an ancient Mediterranean evolutionary centre" (KLAUS 1989). Recently, several morphological traits of this species were revealed to be extremely variable, probably because of the differing ecological

conditions (CLIMENT-MALDONADO et al. 1996).

Pinus canariensis was introduced to Palestine in 1930 (TEAR 1930), and the relatively small plantations in Israel grow under very different climatic, bedrock and soil conditions from those of the Canary Islands. The Canary Island pine recently drew renewed interest in Israel, for possible use in the reclamation of burned forest areas, because of its resilience to fire; post-burn canopy recovery is evident in older trees. This resilience can be attributed to preformed buds which are well insulated by bark and, therefore, can survive a fire; a rare capability among pine species and other conifers. It was, therefore, decided to conduct a systematic study of fresh source material in the Canary Islands, with emphasis on the genetic diversity and field trials performance similar to earlier systematic studies on the genetic diversity of Pinus halepensis Mill. (SCHILLER et al. 1986; GRUNWALD et al. 1986), Pinus brutia Ten. (CONKLE et al. 1988; KARA et al. 1997) and Cupressus sempervirens L. (KOROL et al. 1997; SCHILLER & KOROL 1997) introduced in the 1930's and the geographic origin of the seed sources used in the past is unknown.

Only a few studies have been published on genetic aspects of *P. canariensis*, all of them concerned with the phylogenetic relationships between it and other pine species (*e.g.*, PEDERICK 1970; PRUS-GLOWACKI *et al.* 1985; STRAUSS & DOERKSEN 1990; PIOVESAN *et al.* 1993; KRUPKIN *et al.* 1996). Therefore, the aim of the present study was to analyze the level and structure of genetic diversity within *P. canariensis*, and to relate the data to ecological factors. Knowledge of the genetic diversity and structure within and between islands or ecological zones should greatly assist the introduction of this species into new environments.

MATERIALS AND METHODS

Seed Materials

Twenty-three natural populations and one of artificial origin (Arafo on Tenerife) were included in this study

Island	Pop	pulation	Ν	Ecol. zone	Altitude	TWI	AR	SR	WSurp	MT
Tenerife	0	Arafo	22	1C	1800	0.00				
	1	La Oratava	20	1A	1400	16.95	1175	63.5	653.8	12.6
	2	La Guancha	24	1A	1400	3.63	940	36.4	449.4	13.1
	3	Garachico	26	1A	1350	3.99	810	21.8	502.9	14.1
	4	Vilaflor	32	1B	1850	-6.50	491	2.7	243.1	13.6
	5	Adeje	22	1B	2100	-6.50	453	2.5	261.7	12.4
	6	Arico	31	1 B	1450	-6.75	380	2.1	130.3	12.9
	7	Candelaria	14	1C	1350	-0.30	1214	19.6	846.7	13.8
	8	La Esperanza	15	1C	1200	12.40	1328	71.7	883.6	12.5
	9	La Laguna (Risco de los Pinos)	9	SAP-A	500	0.00	527			
La Palma	10	Punta Llama	22	2A	1850	0.00	847	15.0	593.7	16.1
	11	Barlovento	12	2A	1900	0.00	877	15.8	649.8	15.8
	12	Garafia	36	2A	1450	5.69	948	16.8	683.7	17.0
	13	Punta Gonda	22	2B	800	-0.90	668	11.8	337.3	17.5
	14	El Paso	30	2B	1100	-3.15	828	7.5	635.2	14.4
	15	Fuencaliente	29	2B	1200	-1.10	851	8.1	473.6	14.6
El Hierro	16	San Salvador (Valverde)	30	3	900	-0.42	528	0	258.8	16.3
	17	Risco de las Playas (Valverde)	7	3	900	0.48				
Gran Canaria	18	Tamadaba (Agaete)	27	4	1100	2.90	544	12.0	298.9	16.6
	19	Tirma (Artenara)	28	4	1000	-2.60	478	10.5	243.4	16.8
	20	Tejeda (Las Ninas)	30	4	1000	-6.50	446	1.8	245.5	15.9
	21	Mogan (Los Quemados)	31	4	950	-6.18	271	1.6	108.0	19.0
Gomera	22	Garabuto (Vallehemoso)	11	SAP-B	500	0.00	416			19.7
	23	Imada (Alajero)	5	SAP-B	1180	-0.33				

Table 1. Location.	sample size and	ecological	characteristics of	population studied.
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N = number of trees sampled; SAP = Limited area provenance; TWI = Trade Winds Influence; AR = Average rainfall (mm); SR = Average summer rainfall (mm); WSurp = Average annual water surplus (mm); MT = mean temperature (°C).

(Table 1). The populations were chosen to represent all the provenance regions and sub-regions defined for the species (CLIMENT-MALDONADO *et al.* 1996). The ecological parameters (Table 1) are based, with modifications, upon those calculated by BLANCO-ANDRAY *et al.* (1989).

Open-pollinated seeds were obtained in summer 1996 by single-tree cone collection, collecting from as many trees as possible at any given site (Table 1). Cones were collected from trees separated by more than 100 meter. The target sample size of 35 trees per population was not attained in some populations, because of local physical constraints or scarcity of cones.

Cones were opened and seeds were extracted at the School of Forestry of the Polytechnic University in Madrid, Spain. Part of the seed material available was used for the analysis of diversity (isoenzymes) and part for the establishment of provenance trials.

Electrophoresis

Seeds were germinated on moistened filter paper in Petri dishes at +20 °C. Extraction of enzymes and horizontal starch gel electrophoresis were performed according to CONKLE *et al.* (1982) with some modifications (Table 2). Analyses were performed using eight haploid megagametophytes per mother tree, which gave

Table 2. Buffer systems used and enzymes analyzed.

the probability of $(1-0.5^{8-1}) = 0.992$ of detecting a heterozygous tree. The maternal tissue was homogenized in a grinding plate (KELLEY & ADAMS 1977) together with 75 ml of 0.2 M phosphate buffer pH 7.5, 0.1% Triton X-100, 1% BSA, 0.1% b-mercaptoethanol, for all enzyme systems.

Statistics

The IBM PC version 1.7 of the BIOSYS-1 computer program for the analysis of allelic variation (SWOFFORD & SELANDER 1981) was used to calculate parameters of intra- and interpopulation genetic diversity. The parameters calculated were: mean sample size per locus, mean number of alleles per locus, percentage of polymorphic loci, mean heterozygosity expected from Hardy-Weinberg proportions, and estimates of genetic differentiation and genetic distances.

Genetic diversity among populations was estimated by F-statistics (WRIGHT 1965). F_{ST} was estimated in local populations, in the five islands, in the ecological units and elevation groups, and at species level. Fis, which is a measures of the deficiency of heterozygotes within populations, was calculated for each population and for the species as: $F_{IS} = 1 - (H_{obs}/H_{exp})$. F_{IT} , which represents the level of deviation from Hardy-Weinberg equilibrium in the total population was calculated as: $1 - F_{IT} = (1 - F_{IS}) * (1 - F_{ST})$ (WRIGHT 1965; NEI 1977).

Buffer system		Enzyme systems	Abbreviation	E.C.
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	Alcohol dehydrogenase Alalnine aminopeptidase α-Esterase Leucine aminopeptidase Menodian reductase	ADH AAP EST LAP MNR	1.1.1.1 3.4.11.2 3.1.1.1 3.4.11.1 1.6.99.2
II	Gel buffer: 0.01M tris, 0.005M citric acid, $pH = 8.8$ Electrode buffer: 0.05M NaOH, 0.3M boric acid, $pH = 8.0$	Catalase Glutamate oxaloacetate transaminase	CAT GOT	1.11.1.6 2.6.1.1
III	Gel buffer: 0.002M citric acid, adjusted with morpholine, $pH = 6.1$ Electrode buffer: 0.04M citric acid, adjusted with morpholine, $pH = 6.1$	Aconitase Isocitric dehydrogenase 6-Phosphogluconate dehydrogenase Shikimate dehydrogenase	ACO IDH 6PGD SKDH	4.2.1.3 1.1.1.42 1.1.1.44 1.1.1.25
IV	Gel buffer: 0.002M citric acid, adjusted with morpholine, $pH = 8.3$ Electrode buffer: 0.04M citric acid, adjusted with morpholine, $pH = 8.3$	Acid phosphatase Glutamate dehydrogenase Malate dehydrogenase Peptidase Superoxid dismutase	ACP GDH MDH PEP SOD	3.1.3.2 1.4.1.3 1.1.1.37 3.4.13.1 1.15.1.1

Note: Gels stained for MNR showed five zones of activity. The three slow-migrating zones were interpreted as NDH (HUSSENDORFER *et al.* 1995).

The data on allele frequencies were also used to calculate the proportion of total diversity among subpopulations (G_{ST}) (NEt 1973, 1978), the total gene differentiation (H_r) and the within-population differentiation (H_s) : $G_{ST} = D_{ST} / H_r$. G_{ST} values were calculated for each locus over all populations. NEI's (1978) corrections for small sample sizes were applied.

The GLM procedure was used for one-way analysis of variance (ANOVA) or multiple analysis of variance (MANOVA) to reveal possible relations among the geoclimatic data presented in Table 1, and the results of the statistical analysis of allele frequencies gained from the isoenzyme electrophoresis.

RESULTS

Sixteen enzyme systems with 32 loci were resolved: 28 of them (87.5%) were polymorphic in at least one population; the *Idh*, *Mdh–1*, *Mdh–2* and *Mnr–4* loci were monomorphic in all populations analyzed. Two alleles were resolved in six loci (*Adh–2*, *Cat–2*, *Gdh*, *Pep–1*, *6Pgd–1* and *Sod*), three in 13 loci (*Aap*, *Aco*, *Adh–1*, *Got–1*, *Got–2*, *Lap–1*, *Mdh–4*, *Mnr–3*, *Mnr–5*, *Pep–2*, *Pep–3*, *Skdh–1* and *Skdh–2*), four in six loci (*Est*, *Got–3*, *Lap–2*, *Mnr–2*, *6Pgd–2*, and *6Pgd–3*), five in two loci (*Acp* and *Mdh–3*), and six in the *Mnr–1* locus only (see note at Table 2) (see Tables and Figure in the appendix).

Island	Pop	oulation	Ν	N_p	Α	A_p	Р	H_o	H_{e}	U	$P_{(l)}$
Tenerife	0	Arafo	18	17	1.75	2.41	0.531	0.118	0.128	1	0.028
	1	La Orotava	17	17	1.69	2.29	0.531	0.119	0.114	1	0.029
	2	La Guancha	15	14	1.69	2.57	0.438	0.148	0.125	0	0.000
	3	Garachico	23	17	1.88	2.65	0.531	0.129	0.138	2	0.032
	4	Vilaflor	31	18	1.91	2.56	0.563	0.093	0.123	1	0.048
	5	Adeje	21	15	1.69	2.47	0.469	0.077	0.098	1	0.024
	6	Arico	30	21	2.00	2.48	0.656	0.128	0.139	1	0.017
	7	Candelaria	13	9	1.38	2.33	0.281	0.070	0.079	0	0.000
	8	La Esperanza	12	18	1.69	2.22	0.563	0.130	0.149	2	0.042
	9	La Laguna	9	14	1.63	2.43	0.438	0.139	0.149	1	0.056
Mean				16	1.73	2.44	0.500	0.115	0.124	1	0.028
s.d.				3	0.17	0.13	0.101	0.026	0.022	1	0.019
La Palma	10	Punta Llana	18	18	1.84	2.44	0.563	0.141	0.137	0	0.000
	11	Barlovento	10	16	1.72	2.47	0.500	0.147	0.149	4	0.063
	12	Garafia	27	19	1.78	2.24	0.534	0.105	0.116	2	0.028
	13	Punta Gorda	21	21	1.94	2.43	0.656	0.112	0.143	4	0.030
	14	El Paso	33	19	1.78	2.24	0.594	0.101	0.103	2	0.017
	15	Fuencaliente	28	17	1.78	2.24	0.531	0.125	0.137	0	0.000
Mean				19	1.81	2.34	0.578	0.122	0.131	2	0.023
s.d.				2	0.08	0.11	0.073	0.019	0.017	2	0.024
Gran	18	Tamadaba	21	16	1.78	2.56	0.500	0.106	0.109	4	0.042
Canaria	19	Tirma	27	17	1.78	2.47	0.531	0.169	0.144	3	0.136
	20	Tajeda	28	15	1.84	2.80	0.469	0.113	0.118	4	0.022
	21	Mogen	30	16	1.88	2.69	0.500	0.131	0.137	4	0.025
Mean				16	1.82	2.63	0.500	0.130	0.126	4	0.056
s.d.				1	0.05	0.14	0.025	0.028	0.015	1	0.054
Gomera	22	Garabato	7	11	1.41	2.22	0.344	0.076	0.120	2	0.145
El Hierro	16	San Salvador	29	16	1.84	2.69	0.500	0.105	0.122	4	0.030
Overall Mea	an		21	16	1.76	2.45	0.511	0.117	0.125	2	0.037
s.d.			8	3	0.15	0.17	0.098	0.026	0.019	1	0.038

Table 3.	Diversity	statistics for	r <i>Pinus</i>	canariensis	populations.
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N = Number of individuals analyzed; $N_p =$ number of polymorphic loci; A = number of alleles per locus; $A_p =$ number of alleles per polymorphic locus; $H_o =$ observed hetorozygosity (direct count); $H_e =$ expected hetorzygosity (unbiased estimate); U =number of unique alleles to that population; $P_{(1)} =$ average frequency of private alleles; P = proportion of polymorphic loci.

Genetic diversity parameters in 22 populations growing on the five islands are presented in Table 3. The mean number of alleles per locus (A) was 1.76 ranging from 1.38 to 2.00. The mean number of alleles per polymorphic locus (A_n) was 2.45 ranging from 2.22 to 2.80. The mean proportion of polymorphic loci (P), at the 0.99 criterion, was 0.511 ranging from 0.281 to 0.656. The mean observed heterozygosity (H_{abs}) was 0.117 ranging from 0.070 to 0.169, and the mean expected heterozygosity (H_{exp}) was 0.125 ranging from 0.079 to 0.149. There are differences between populations on the same island, e.g., the observed and expected heterozygosity of the Adje and Candelaria populations on Tenerife, and the El Paso population on La Palma are much lower than one s.d. of the average for all populations on the same island (Table 3). The highest level of Islands mean observed heterozygosity was found on the island of Gran Canaria (0.130), and the lowest - on Gomera (0.076). The lowest observed heterozygosity was found in the Garabato population on the Island of Gomera (0.076); which can be explained as sampling error (only 7 trees sampled). Differences between the islands in the number of unique (= private) alleles in the populations and their frequency where revealed, e.g., the island of Gran Canaria has the largest number of unique alleles in each of the population; the only one population on the island of Gomera has the highest frequency of unique alleles and the lowest observed and expected heterozygosity.

Differences among the islands, in the means of the statistical parameters, are much smaller. Differences among groupings of populations, according to elevation (500-1000 m, 1001-1500 m and 1501-2100 m) or to ecological zone (Table 1), were negligible except for the differences in the numbers of unique (= private alleles) to each population. Populations growing between 500 and 1000 m a. s. l. have larger numbers of private alleles than those growing at higher elevations. Analysis of genetic diversity according to the three altitude zones is difficult because the distribution of populations among the zones differs among the islands. Thus, in the lowest altitude zone, which displays the highest number of private alleles, there is only one population from Tenerife (the island with the lowest number of private alleles), whereas, on the contrary, most populations in the highest altitude zone are from this island. The possibility that lower-altitude populations have evolved more independently from one another than higher-altitude ones is of interest; it would be consistent with the topography of the islands, which facilitates easier connections among populations at higher altitudes than among those lower down. In addition, most forest fires tend to affect the forests at lower elevations, thus leading to differing evolutionary

Table 4. Chi-square analysis across all populations of *P. canariensis.*

Locus	Number of alleles	χ ²	р	d.f.
Aap	3	44.594	0.363	42
Aco	3	164.599	0.000	42
Acp	5	657.268	0.000	84
Adh–1	3	177.037	0.000	42
Adh–2	2	30.649	0.079	21
Cat	2	227.683	0.000	21
Est	4	199.655	0.000	84
Gdh	2	34.056	0.035	21
Got–1	3	46.985	0.276	42
Got-2	3	102.360	0.000	42
Got-3	4	96.775	0.161	84
Lap–1	3	124.659	0.000	42
Lap–2	4	149.192	0.000	63
Mdh–3	5	163.827	0.000	84
Mdh–4	3	39.785	0.568	42
Mnr–1	6	231.705	0.000	105
Mnr–2	4	97.501	0.003	63
Mnr–3	3	50.524	0.172	42
Mnr–5	3	124.403	0.000	42
Pep-1	2	22.185	0.389	21
Pep-2	3	91.938	0.000	42
Pep-3	3	108.108	0.000	42
6Pgd–1	2	68.662	0.000	21
6Pgd–2	4	212.795	0.000	84
6Pgd–3	4	306.399	0.000	63
Skdh–1	3	192.073	0.000	42
Skdh–2	3	55.206	0.083	42
Sod	2	32.514	0.052	21
Total		3852.6	2.184	1386

sequences among neighboring populations. Thus, the fact that private alleles differ among populations within the same ecological zone supports the idea that these populations have evolved differently.

Hetrogenity chi-squre analysis of the polymorphic loci in *P. canariensis* at species level are presented in Table 4. At most of the loci, one allele predominates in nearly all populations whereas the alternative alleles vary greatly in their frequencies (see table of allele frequencies in the appendix). Contingency chi-square analyses for allele frequency differences among populations showed that the differences were significant for 19 out of the 28 polymorphic loci, at the level of p< 0.05. Only the nine loci: *Aap*, *Adh*-2, *Got*-1, *Got*-3, *Mdh*-4, *Mnr*-3, *Pep*-1, *Skdh*-2 and *Sod* did not differ much in their allele frequencies among populations.

Table 5 shows estimated parameters of genetic diversity: F_{ST} , the coefficient of genetic diversity between populations, in comparison with the genetic variation attributable to differences within populations

Locus	F_{IS}	F_{IT}	F_{ST}	H_s	H_t	G_{ST}
Aap	0.101	0.133	0.036	0.056	0.058	0.036
Aco	0.009	0.126	0.117	0.246	0.278	0.117
Acd	0.021	0.155	0.136	0.460	0.533	0.136
Adh–1	-0.038	0.690	0.103	0.408	0.455	0.103
Adh-2	-0.038	-0.005	0.032	0.009	0.009	0.032
Cat	-0.273	0.047	0.251	0.032	0.043	0.251
Est	0.299	0.346	0.067	0.314	0.337	0.067
Gdh	-0.045	-0.005	0.038	0.010	0.011	0.038
Got-1	-0.041	-0.017	0.023	0.048	0.050	0.044
Got-2	0.149	0.201	0.061	0.070	0.076	0.081
Got-3	-0.061	-0.013	0.045	0.135	0.141	0.043
Lap-1	0.696	0.726	0.096	0.015	0.017	0.096
Lap-2	0.005	0.060	0.055	0.173	0.183	0.055
Mdh–3	0.087	0.143	0.061	0.215	0.230	0.061
Mdh-4	-0.046	-0.016	0.029	0.033	0.034	0.029
Mnr–1	0.000	0.044	0.044	0.126	0.132	0.044
Mnr-2	0.015	0.077	0.063	0.436	0.465	0.063
Mnr–3	0.075	0.103	0.031	0.041	0.042	0.031
Mnr-5	0.047	0.150	0.108	0.443	0.497	0.108
Pep-1	-0.028	-0.004	0.024	0.007	0.008	0.024
Pep-2	0.202	0.258	0.071	0.094	0.101	0.075
Pep-3	0.444	0.481	0.065	0.027	0.029	0.072
6Pgd-1	0.786	0.807	0.098	0.018	0.020	0.098
6Pgd-2	-0.107	-0.036	0.064	0.119	0.127	0.064
6Pgd-3	-0.061	0.074	0.127	0.257	0.294	0.127
Skdh–1	-0.055	0.069	0.118	0.111	0.126	0.118
Skdh-2	-0.044	-0.004	0.038	0.003	0.003	0.038
Sod	0.002	0.002	0.035	0.003	0.003	0.035
Total	0.041	0.129	0.091	0.122	0.134	0.091

Table 5. Genetic characteristics estimates for 28 polymorphic loci in *P. canariensis*.

(F_{IS}), and the level of deviation from Hardy-Weinberg equilibrium in all population (F_{IT}). The high level of heterozygotes deficiency (F_{IT}) in *Aap*, *Est*, *Got–2*, *Pep–2*, *Pep–3*, *Lap–1*, and *6Pgd–1* loci is expressed only due to deficiency of heterozygotes within populations (F_{IS}). For the polymorphic loci, F_{ST} ranged from 0.024 (*Pep–1*) to 0.251 (*Cat*), F_{ST} at species level was 0.091, *i.e.*, 9% of the total intraspecific variation resulted from interpopulation variation.

Figure 1presents a comparison between the popula tions of Wright's fixation index (F_{IS}). The figure shows heterozygote deficiency for 17 out of the 22 populations; chi-square test according to LI and HOROVITZ (1953) revealed that all excesses and/or deficiencies of heterozygotes within the 22 population were non-significant.

Summaries of the genetic structure parameters at the levels of islands, elevation, ecological zones and populations are shown in Table 6. The table shows that each island is unique in its genetic structure, which is due to the large differences in the numbers of



Figure 1. Population F_{IS} index.

monomorphic loci on each of the islands (see Table in the appendix). On Tenerife (189 trees), in addition to the four monomorphic loci listed above (Idh, Mdh-I, Mdh-2 and Mnr-4), three additional loci (Gdh, Lap-1) and Sod) were monomorphic; on La Palma (137 trees), two additional loci (Pep-1, 6Pgd-1); on Gran Canaria (106 trees), five additional loci (Got-2, Lap-1, 6Pgd-1, Skdh-2 and Sod); on the island of El Hierro (29 trees), 12 additional loci (Aap, Adh-2, Cat, Gdh, Lap-1, Mnr-3, Pep-1, Pep-3, 6Pgd-1, Skdh-1, Skdh-2, Sod), *i.e.*, more than 50% of the total; and on the island of Gomera (7 trees), 18 additional loci (Aap, Adh-2, Cat, Gdh, Lap-1, Mdh-3, Mdh-4, Mnr-1, Mnr-3, Pep-1, Pep-2, Pep-3, 6Pgd-1, 6Pgd-2, 6Pgd-3, Skdh-1, Skdh-2, Sod) were monomorphic, *i.e.*, more than 67% of the total.

Differences in the genetic structures of P. canariensis populations on all the islands, defined according to elevation of the sites above sea level are also presented in Table 6. These differences are also due to the large differences in the numbers of monomorphic loci characterizing each altitudinal grouping. All the eight populations (122 trees) growing at elevations between 500 and 1000 m, (mean = 819 m a.s.l.) are, in addition to the four monomorphic loci mentioned above, also monomorphic at the Gdh, Lap-1, Pep-1 and Sod loci, whereas the 11 populations (219 trees) growing at elevations between 1000 and 1500 m (mean = 1290 m a.s.l.) had only one additional monomorphic locus, namely Skdh-2. At higher altitudes, between 1500 and 2100 m (mean = 1900 m a.s.l.) the five populations (98) trees) belonging to this group were characterized by the addition of four monomorphic loci, namely Adh-2, Pep-1, 6Pgd-1 and Sod.

Table 6 also shows the genetic structure parameters according to the ecological units on the two largest islands, namely, Tenerife with three different ecological units, and La Palma with two ecological units (CLIMENT-MALDONADO *et al.* 1996). Populations within each of the ecological units differ from those within

Island	Population	No of trees	<i>F</i> _{<i>IS</i>} *	No of populations	F _{IS} *	F _{IT}	F _{ST}
A. At Island le	vel						
Tenerife La Palma Gran Canaria				10 6 4	0.041 0.047 -0.049	0.113 0.096 0.037	0.074 0.051 0.085
Gomera El Hierro				1	0.320 0.130	0.320 0.130	
B. According t	o altitude; Altituc	le between 5	00 and 1000	m a.s.l.		······	
Tenerife	La Laguna]	9	0.073				
La Palma El Hierro	Punta Gorda San Salvador	21	0.013				
Gran Canaria	Tirma	27	-0.074				
	Tejeda	28	0.013				
	Mogan	30	0.021				
Gomera	Garabato	7	0.320				
				.7	0.056	0.143	0.092
Altitude betwee	en 1001 and 1500	m a.s.l.					
Tenerife	La Orotava	17	-0.031				
	La Guancha	15	-0.157				
	Garachico	23	0.014				
	Arico	30	0.031				
	Candelaria	13	0.069				
1	La Esperanza	12	0.051				
La Palma	Garafia	27	0.064				
	El Paso Euonocliento	22	0.010				
Gran Canaria	Tamadaba	28	0.034				
				10	0.017	0.097	0.081
Altitude betwee	en 1501 and 2100	m a.s.l.		<u> </u>			
 Tenerife	Arafo	18	0.044			<u> </u>	
101101110	Adeje	21	0.122				
	Vilaflor	31	0.118				
La Palma	Punta Llana	18	-0.003				
	Barlovento	10	-0.106		·		
				5	0.061	0.125	0.068
C. According	to islands and eco	logical zone	S				
Tenerife 1A	La Orotava	17	-0.031				
	La Guancha Garachico	15 23	-0.157 0.014				
				3	-0.048	-0.007	0.039
Tenerife 1B	Arico	30	0.031				
	Adeje Vilaflor	21 31	0.122 0.118				
				3	0 149	0 181	0.038
						0.101	0.050

Table 6. Genetic diversity of P. canariensis according to island, altitude and ecological zonation.

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Island	Population	No of trees	F_{IS}^{*}	No of populations	<i>F_{IS}</i> **	F _{IT}	F _{ST}
Tenerife 1C	Candelaria	13	0.069				
	La Esperanza	12	0.051				
	Arafo	18	0.044				
				3	0.087	0.167	0.088
La Palma 2A	Punta Llana	18	-0.003				
	Barlovento	10	-0.106				
	Garafia	27	0.064				
				3	-0.004	0.046	0.050
La Palma 2B	El Paso	33	0.016				
	Funcaliente	28	0.034				
	Punta Gorda	21	0.013				
				3	0.101	0.123	0.024

Table 6. Genetic diversity of P	. canariensis according to island	, altitude and ecologica	l zonation (continued).
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N = number of trees analyzed; F_{IS}^* = genetic diversity within populations; F_{IS}^{**} = within-group genetic diversity; F_{TT} = total genetic diversity; F_{ST} = proportion of total diversity among populations.

other units on the same island in their number of additional monomorphic loci. On Tenerife, in addition to the monomorphic loci characterizing this island (Idh, Mdh-1, Mdh-2, Mnr-4, Gdh, Lap-1 and Sod), the three populations within the ecological zone 1.A (55 trees) were also monomorphic at the Cat, Pep-3, 6Pgd-1 and Skdh-2 loci, the three populations within the ecological zone 1.B (82 trees), at the Adh-2, Got-3, and Pep-3 loci, and the three populations within the 1.C ecological zone (43 trees), at the Adh-2, Cat, Got-3, Mdh-4, Mnr-3, Pep-1, and Skdh-2 loci. On La Palma, in addition to the monomorphic loci characterizing this island's populations (Idh, Mdh-1, Mdh-2 Mnr-4, Pep-1 and 6Pgd-1), the three populations within the ecological zone 2.A (55 trees), are characterized by the monomorphic loci, Adh-2, 6Pgd-2 and Skdh-2, and the three populations of zone 2.B (82 trees) by the monomorphic loci Gdh. Got-1 and Sod.

Table 7 summarize several significant linear relations among the geoclimatic parameters by using the ANOVA and MANOVA procedures. Furthermore, linear and significant relations were revealed between the geoclimatic parameters and several genetic statistical parameters. Table 8 shows regressions with significant negative correlations between the mean number of alleles per locus (A) and the mean number of alleles per polymorphic locus (Ap) with the water-surplus (WSurp); and between Ap and the average annual rainfall, *i.e.*, as water availability declines the average number of alleles per locus or per polymorphic locus in the population rises. Furthermore, a linear regression

Table 7. Correlation coefficients (r) and probability (p) of regressions between geoclimatic data measured at the site of the different provenances of *P. canariensis*.

Variable	by variable	Correlation coefficient (r)	Р
TWI	ALT	0.061	0.7861
AR	ALT	0.254	0.2672
AR	TWI	0.748	0.0001
SR	ALT	0.024	0.9222
SR	TWI	0.900	0.0000
SR	AR	0.801	0.0000
WSurp	ALT	0.185	0.4473
WSurp	TWI	0.656	0.0023
WSurp	AR	0.956	0.0000
WSurp	SR	0.666	0.0018
MT	ALT	-0.568	0.0072
MT	TWI	-0.240	0.0294
MT	AR	-0.482	0.0268
MT	SR	-0.471	0.0416
MT	WSurp	-0.326	0.1732

TWI = trade wind influence; AR = average annual rainfall; SR = average summer rainfall; WSurp = average water surplus; MT = mean annual temperature; ALT = altidute above sea level.

with highly significant correlations, between allele frequencies in the *Lap*-2 and *Aap* loci and the altitude: of the four alleles in the *Lap*-2 locus, the frequency of allele 4 within populations is positively related to altitude; of the three alleles in the *Aap* locus, the

Variable 1 allele	by variable 2	Correlation coefficient (<i>r</i>)	р
А	WSurp	-0.659	0.0021
Ap	WSurp	-0.701	0.0008
Ap	AR	-0.547	0.0102
Aap 2	ALT	-0.618	0.0022
Aap 3	ALT	0.623	0.0020
Adh-1 3	SR	0.617	0.0049
Est 4	SR	0.588	0.0081
Lap-2 = 3	ALT	0.538	0.0099
Mnr-5 1	MT	0.657	0.0011
Mnr-5 2	MT	-0.655	0.0013
<i>F_{IS}</i> of <i>6pgd</i> –2	МТ	-0.624	0.0074

Table 8. Correlation coefficient (*r*) and probability of the regression between genetic statistical parameters or enzyme systems (variable 1) and geoclimatic parameters (variable 2).

TWI = trade wind influence; AR = average annual rainfall; SR = average summer rainfall; WSurp = average water surplus; MT = mean annual temperature; ALT = altidute above sea level; A =- mean number of alleles per locus; Ap =- mean number of alleles per polymorphic locus; AAP, ADH, EST, LAP, MNR, 6PGHD – enzyme systems, F_{tS} = within population genetic diversity.

frequency within populations of allele 2 is negatively, and of allele 3, therefore, positively related to the altitude of the population. MANOVA enabled geocli matic parameters such as Ar, WSurp and Sr to be added to the regression with altitude, thus strengthening the relations between allele frequencies within populations and altitude. Linear regressions with significant positive correlations were found between the average summer rainfall (SR) and the frequencies within populations of the third allele in the Adh-1 locus and the fourth allele in the Est locus; i.e., the greater the average summer rainfall, the higher the allele frequency. There are linear regressions with significant correlations between the frequencies of two alleles in the Mnr-5 locus and the yearly mean temperature (MT); the relations between allele 1 and MT were positive, therefore, the relations of allele 2 were negative. ANOVA revealed linear regressions with significant correlations between allele frequencies in different enzyme systems (Table 9).

Figure 2 shows a dendrogram of the *Pinus canariensis* populations based on the CAVALLI-SFORZA and EDWARDS (1967) chord distance. According to WEST-FALL & CONKLE (1992), the arc and chord distance analysis (CAVALLI-SFORZA & EDWARDS 1967) is better suited for determining the existence of geographic patterns in genetic diversity. The average distance between them was used as a marker to distinguish between groups of populations, and the resulting dendrogram joins, in a geographic sense, all but two (La Esperanza and La Laguna) of the populations of Tenerife. All except two (Punta Llana and Barlovento) of the populations of La Palma are clustered together



Figure 2. Dendrogram based on Cavalli-Sforza and Edwards chord distance of native *P. canariensis* populations.

with the San Salvador population of El Hierro. Three of the four populations of Gran Canaria are clustered together but not on the same branch, with the Punta Llana population of La Palma. The last five populations, each clustered separately, are on different islands. The Arafo population is of artificial origin and its union

Table 9. Relations among loci in their allele frequencies in
Pinus canariensis, and the correlation coefficient of the
linear regression (all correlation presented in this table
have a probability $p < 0.01$).

Variable		by variable		Correlation
Locus	allele	locus	allele	coefficient (r)
Adh–1	3	Acp	2	-0.547
Est	4	Aap	1	-0.641
Got–3	2	Aco	1	0.591
Got–3	3	Aco	1	-0.562
Got–3	2	Aco	2	-0.611
Got–3	3	Aco	2	0.636
Got–3	2	Est	1	-0.699
Got–3	3	Est	1	0.699
Lap-2	3	Aap	3	0.601
Lap-2	3	Aap	2	-0.589
Mdh-3	1	Est	1	0.700
Mdh–3	4	Est	1	-0.561
Mdh-3	1	Got-3	2	-0.747
Mdh–3	1	Got-3	3	0.680
Mdh-3	4	Got-3	2	0.554
Mdh-4	2	Got-3	3	-0.603
Mnr–1	3	Aco	1	0.542
Mnr–1	3	Aco	2	-0.540
Mnr–3	3	Got-3	1	0.587
Pep-2	2	Est	4	-0.577
Pep-2	3	Est	4	0.561
Pep-2	3	Got-2	3	0.561
Pep-2	2	Lap-2	3	-0.633
Pep-2	3	Lap–2	3	0.656
6pgd–3	2	Est	4	-0.543
6pgd–3	3	Est	4	0.572
6pgd–2	4	Got-1	3	0.575
Skdh–1	3	Acp	2	-0.554
Skdh-1	2	6pgd–3	3	-0.637
F_{IS} of Mdh	-3	F _{IS} of Got-1		0.709
F_{IS} of Mnr-	-1	F_{IS} of $Adh-1$		0.651
F_{IS} of Mnr-	-1	F_{IS} of Mdh-4		-0.894
F_{IS} of 6Pgc	<i>1</i> –2	F_{IS} of Mnr-2		0.646
F_{IS} of $6Pga$	<i>l–3</i>	F _{IS} of Mnr-2		-0.660

with the La Guancha population may reflect the seed origin: according to the Canary Island people, this natural forest has been traditionally used as a seed source for plantations.

DISCUSSION

Natural *Pinus canariensis* forests grow on 60.000 ha distributed among five of the seven Canary Islands (CLIMENT-MALDONADO *et al.* 1996), four of which (*i.e.*, Gomera, El Hierro, La Palma and Gran Canaria) are less than 100 km from the central and largest island – Tenerife. This means that the Canary Island pine has a

very limited, naturally disjunct geographic distribution, which is a fraction of the past domain of the species (KLAUS 1989). There is no knowledge of the time, means and order of colonization of these islands by *P. canariensis*; nor of events of ecological significance on a geological time scale, such as volcanic eruptions, and their possible influence on the pine populations on the various islands. Therefore, the analysis of genetic diversity within and among 22 subpopulations of *P. canariensis* growing on five Canary Islands revealed important information about geographic pattern of genetic variation in this species.

The total number of loci in the present study was 32 in all 22 populations and the number of alleles per locus (A) varied from a minimum of 1.38 at Candelaria (Tenerife) to a maximum of 2.00 at Arico (Tenerife) with an overall mean of 1.76 for 22 populations. In comparison with other pine species, P. canariensis was found to have a relatively small number of alleles per locus (A = 1.76%), and a small proportion of polymorphic loci (P = 51.1%) which ranged from 28% to 65%. However, low values of polymorphic loci were observed only in two populations: Candelaria on Tenerife, and Garabato on Gomera, for the other 20 populations this value varied from 44% to 65% (Table 3). For comparison, percentage of polymorphic loci reported for other Mediterranean pine species which probably evolved from the progenitor of toady's P. canariensis (KLAUS 1989) were: 70 and 66, respectively in P. nigra (SCALTSOYIANNES et al. 1994); 50 and 13, respectively in P. halepensis (KOROL & SCHILLER 1996; TEISSEIRE et al. 1995; SCHILLER et al. 1986); and 70 at the 99% criterion in P. brutia (KARA et al. 1997). Mean values of polymorphic characteristics for 15 pine species, were 63% and 2.2 alleles per locus for other pines (HAMRICK et al. 1981) The present results are similar to the levels of genetic variations in other pine species and are located in the middle of the scale of variation

Thirteen of the 28 polymorphic loci had a H_i value of less than 0.10 (Table 5) indicating that the allele common to these loci had a frequency of 0.95 or higher. This level of allele frequencies is the reason for the relatively low H_e value of only 0.125 (Table 3). Generally, wind-pollinated species such as conifers are characterized by very high levels of genetic variation and they are one of the most variable groups of species (HAMRICK *et al.* 1992). However, *P. canariensis* has rather a low level of genetic diversity $H_e = 0.125$ (0.079 – 0.149) in comparison with other Mediterranean pine species such as *P. nigra* (0.274) (NIKOLIĆ & TUCIĆ 1983), *P. sylvestris* in Eastern Europe (0.286) (GONCHARENKO *et al.*, 1994), *P. halepensis* (0.144) (KOROL & SCHILLER 1996), *P. brutia* (0.265) (KARA

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et al. 1997) and with other gymnosperms (0.169) (HAMRICK et al., 1992). This may have resulted from the decrease in effective population size of this specie in the past; at present P. canariensis, occupies only a restricted geographic range, and has a relatively low genetic diversity. According to POLITOV et al. (1992), wind-pollinated conifers have higher mean values of polymorphic characteristics, e.g., the mean number of alleles per locus (A), for 30 different pine species was 2.08, and the expected heterozygosity (H_e) 0.169. According to HAMRICK et al. (1992) and PARKER & HAMRICK (1996), mean values of A and H_e for gymnosperms were 2.38 and 0.169, respectively. Genetic differentiation, H_s of P. canariensis was 0.122, and it ranged from 0.002 to 0.460, whereas the mean H_{c} within other pine species is 0.172, and within gymnosperms is 0.157 (HAMRICK et al. 1992).

The G_{ST} estimations of genetic diversity was characterized by NEI (1973, 1977) as analogous of Wright's F_{ST} , and it measures gene diversity in subdivided population. Deviations from the Hardy-Weinberg equilibrium, *i.e.*, the F_{IS} index, show a slight heterozygote deficiency at the species level (Table 5). Within species, high variability of F_{is} values among loci, from -0.273 at the Cat locus (excess of heterozygosity) to 0.786 at the 6Pgd-1 locus (heterozygotes deficiency) can be seen. Four loci, namely Aco, Lap-2, Mnr-1 and *Mnr*-2, hade a low mean value of F_{is} , indicating that these loci were in stage of equilibrium. The F_{ST} and/or G_{ST} analysis of our results (Table 5) indicates that 90.9% of the genetic variation occur within populations and only 9.1% - among populations. Such low levels of interpopulation diversity are usual in conifers (HAM-RICK & GODT 1989). Levels of F_{ST} for conifers range approximately from 1% to 16%. For example, G_{st} values reported for the Mediterranean P. nigra 13.5% (NIKOLIĆ & TUCIĆ 1983); Aleppo pine 4.3% (KOROL & SCHILLER, 1996) and P. brutia - 5.3% (KARA et al. 1997). Low levels of genetic diversity of allozyme loci between populations can be explained by wind-pollination, seed dispersal by wind and by birds, that reduce the influence of genetic drift and therefore, decreases the heterogeneity of allele frequencies and intergenetic differentiation (HAMRICK et al. 1981; HAMRICK & GODT 1989; HAMRICK et al. 1992). In our relatively low diversity among groups of populations (Islands) we have found a slight decreases in diversity (F_{ST}) , in rather isolated populations on the different islands.

Some of the sampled populations grow under limiting conditions such as high altitude, geographically limited site area, etc., which could have either a positive or a negative influence on the meeting system which is expressed by the F_{IS} and the F_{IT} values at population level. The mean F_{IS} for all the 22 populations was 0.041; it ranged from 0.344 at Garabato on the island of Gomera to -0.157 at La Guancha on Tenerife. The mean F_{IS} of the five populations with excess heterozygosity was -0.074, whereas that of the 17 heterozygosity deficient populations was 0.075; therefore, in the latter 17 populations the observed heterozygosity was lower than the expected heterozygosity. Heterozygote deficiencies have been reported for many predominantly outcrossing species (BROWN 1979; FADY & CONKLE 1993), including pines (DANCIK & YEH 1983; PARKER & HAMRICK 1996), which can be attributed to several factors, such as inbreeding, sampling error, the Wahlund effect and, possibly, microscale genetic differentiation. Whatever the cause, the present results show that there is deficiency of heterozygotes at the island level except on the island of Gran Canaria (Table 6).

Significant relations among geographical and climatological parameters on the Canary Islands, and among these parameters and genetic parameters were revealed applying ANOVA or MANOVA procedures (Table 7, Table 8). Similar results were found by MITTON et al. (1980) in a study of the genetic differentiation along an altitudinal gradient of Pinus ponderosa. Significant relations between altitude and allele frequencies were found also in Pinus brutia (KARA et al. 1997), which supported earlier conclusions about genetic differentiation along an altitudinal gradient based on morphological traits (ISIK 1993). In a review paper, WESTFALL & CONKLE (1992) showed that canonical analysis between several geographical parameters and multi-locus scores indicate significant geographic variation. "Allozymes in tandem with other traits, are useful in development of breeding zones". The climate, e.g., trade wind influence, which also depends on the orography, influences significantly the water availability at *P. canariensis* sites on the Canary Islands which varies between very wet to extremely dry. SCHILLER & WAISEL (1989) showed that the expected heterozygosity of different P. halepensis provenances is significantly related to the mean annual rainfall. Similarly to native natural Aleppo pine in the East Mediterranean, were the climatic factors referred to are water availability and temperature regime, which change strongly over short distances, the same on the Canary Islands. The relations between genetic parameters as revealed in Table 8 and geoclimatic parameters are in agreement with the results of NEVO (1983), who postulated that isoenzyme variation is partly related to, and can be predicted by, climatic and biotic structure.

In conclusion, the present study revealed variation in the genetic composition of *P. canariensis*, which can be attributed to differences among the islands, and geoclimatic influences. This variation must be taken into consideration when seed distribution zones are established on the Canary islands. The suitability of seed sources for over-seas introduction must be tested in introduction trials.

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REFERENCES

- BLANCO- ANDRAY, A., CASTROVIEJO- BOLOBAR, M., FRAILE-SANCHEZ, J. L., GANDULLO-GUTIERREZ, J. M., MUNOZ DE LA FUENTE, L. A. & SANCHEZ- PALOMARES, O. 1989:
 Estudio Ecologica del Pino Canario. Serie Tecnica No. 6, 195 pp. Instituto Nacional para Conservacion de la Naturaleza, Madrid, Spain.
- BRAMWELL, D. 1976: The endemic flora of the Canary Islands: distribution, relationships and phytogeography. *In* Biogeography and ecology on the Canary Islands. Edited by Kunkle, G. Dr. W. Junk, b.v. Publish. The Hague. pp. 207–239.
- BROWN, A. H. D. 1979: Enzyme polymorphism in plant populations. *Theor. Pop. Biol.* 15: 1-44.
- CAVALLI- SFORZA, L. L. & EDWARDS, A. W. F. 1967: Phyloganatic analysis: models and estimation procedures. *Evolution* 21: 550–570.
- CLIMENT- MALDONADO, J., GIL- SANCHEZ, L. & DE TURO-DE-REYNA, M. 1996: Las regiones de procedencia de *Pinus* canariensis Chr. Sm. ex DC. Instituto Nacional Para Conservacion de la Naturaleza, Madrid, Spain, 50 pp.
- CONKLE, M. T., HODGSKISS, P. D., NUNNALLY, B. L. & HUN-TER, S. C. 1982: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, General Technical Report, PSW-64.
- CONKLE, M. T., SCHILLER, G. & GRUNWALD, C. 1988: Electrophoretic analysis of diversity and phylogeny of *Pinus* brutia and closely related taxa. Sys. Bot. 13: 411–424.
- CRITCHFIELD, W. B. & LITTLE, E. L., JR. 1966: Geographic Distribution of the Pines of the World. USDA. For. Serv. Miscellaneous Pub. 991.
- 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.
- FADY, B. & CONKLE, M. T. 1993: Allozyme variation and possible phylogenetic implications in *Abies cephalonica* Lound and some related eastern Mediterranean firs. *Silvae Genet.* 42: 351–359.
- FERNANDOPULLE, D. 1976: Climatic characteristics of the Canary Islands. In: Biogeography and Ecology on the Canary Islands. Edited by Kunkle, G. Dr. W. Junk, b.v. Publish. The Hague. pp. 185–206.

GONCHARENKO, G. G., PADUTOV, V. E. & SILIN, A. E. 1993:

Allozyme variation in natural populations of Eurasian pines. I: Population structure, genetic variation & differentiation in *Pinus pumila* (Pall.) Regel from Chukots and Sakhalin. *Silvae Genet.* **42**: 237–246.

- GRUNWALD, C., SCHILLER, G. & CONKLE, M. T. 1986: Isozyme variation among native stands and plantations of Aleppo pine in Israel. *Israel J. Bot.* 35: 161–174.
- HAMRICK, J. L. & GODT, M. J. 1989: Allozyme diversity in plant species. *In*: Plant population genetics breeding and genetic resources. Edited by A. H. D. Brown, M. T. Clegg and Kahler, J. L. Sinauer Associates, Sunderland. MA. pp. 43–63.
- HAMRICK, J. L., GODT, M. & SHERMAN-BROYLES, S. L. 1992: Factors influencing levels of genetic diversity in woody plant species. *In*: Population Genetics of Forest Trees. Edited by W. T. Adams, S. H. Strauss, D. L. Copes & A. R. Griffin. Kluwer Academic Publishers, London. pp. 95–125.
- HAMRICK, J. L., MITTON, J. B. & LINHART, Y. B. 1981: Levels of genetic variation in trees: Influence in life history characteristics. *In*: Conkle, M.T. (ed), Isozyme in North American Trees and Forest Insects, pp 4–35. USDA Forest Servise, *Gen Tech Rep, PSW*.
- HUSSENDORFER, E., KONNERT, M. & BERGMANN, F. 1995: Inheritance and linkage of isozyme variants of silver fir (*Abies alba* Mill.). Forest Genetics 2: 29-40.
- ISIK, K. 1986: Altitudinal variation in *Pinus brutia* Ten.: Seed and seedling characteristics. *Silvae Genet.* 35: 58–67
- KARA, N., KOROL, L., ISIK, K. & SCHILLER, G. 1997: Genetic diversity in *Pinus brutia* Ten.: Altitudinal variation. *Silvae Genet.* 46: 155–161.
- KASAPLIGIL, B. 1978: Past and present pines of Turkey. *Phytologia* **40**: 99–153.
- KELLEY, W. A. & ADAMS, R. P. 1977: Preparation of extracts from juniper leaves for electrophoresis. *Phytochemistry* 16: 513–516.
- KLAUS, W. 1989: Mediterranean pines and their history. Pl. Sys. Evol., 162: 133–163.
- KOROL, L., KARA, N., IŞIK, K. & SCHILLER, G. 1997: Genetic differentiation among and within natural and planted *Cupressus sempervirens L*. Eastern Mediterranean populations. *Silvae Genet.* 46: 152–155.
- KOROL, L. & SCHILLER, G. 1996: Relations between native Israeli and Jordanian Aleppo pine (*Pinus halepensis* Mill.) based on allozyme analysis: a note. *Forest Genetics* 3: 197–202.
- KRUPKIN, A. B., LISTON, A. & STRAUSS, S. H. 1996: Phylogenetic analysis of the hard pine (*Pinus* subgenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. *Amer. J. of Bot.* 83:489–498.
- LI, C. C. & HOROVITZ, D. G. 1953: Some methods of estimating the inbreeding coefficient. Am. J. Hum. Genet., 5: 107–117.
- MERCER, J. 1980: Geology, topography, climate, flora and fauna. *In:* The Canary Islanders; their prehistory conquest and survival. Rex Collins, London. pp. 4–13.
- MILLAR, C. I., STRAUSS, S. H., CONKLE, M. T. & WESTFALL, R. D. 1988: Allozyme differentiation and biosystematics of the Californian closed-cone pines (*Pinus subsec. Oocarpae*). Systematic Botany **13**: 351–369.
- MIROV, N. T. 1967: The Genus Pinus. The Ronald Press Co.

New York.

- MITTON, J. B., STURGEON, K. B. & DAVIS, M. L. 1980: Genetic differentiation in ponderosa pine along a steep elevational transect. Silvae Genet. 29: 100–103.
- NEI, M. 1973: Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA. 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. 1983: Population genetics and ecology: the interface. *In*: D.S. Bendall, (Ed), Evolution from Molecules to Men. Cambridge University Press, Cambridge, pp. 223–272.
- NIKOLIĆ, D. & TUCIĆ, N. 1983: Isoenzyme variation within and among populations of European black pine (*Pinus nigra* Arnold). *Silvae Genet*. **32**: 3–4.
- PARKER, K. & HAMRICK, J. L. 1996: Genetic variation in sand pine (*Pinus clausa*). *Can. J. For. Res.* **26**: 244–254.
- PEDERICK, L. H. 1970: Chromosome relationships between *Pinus* species. *Silvae Genet.* **19**: 171–180.
- PIOVESAN, G., PELOSI, F., SCHIRONE, A. & SCHIRONE, B. 1993: Taxonomic evaluations of the genus *Pinus* (Pinaceae) based on electrophoretic data of salt soluble and insoluble seed storage proteins. *Pl. Sys. Evol.* 186: 57–68.
- POLITOV, D. V., KRUTOVSKII, K. V. & ALTUKHOV, YU. P. 1992: Isozyme loci characteristics of gene blanks of populations of cedar pines. *Genetika* 28: 93–114. [In Russian].
- PRUS- GŁOWACKI, W., SZWEYKOWSKI, J. & NOWAK, R. 1985: Serotaxonomical investigation of the European pine species. *Silvae Genet.* 34: 162170.

SCALTSOYIANNES, A., ROHR, R., PANETSOS, K. P. & TSAKT-

SIRA, M. 1994: Allozyme frequency distributions in five European populations of black pine (*Pinus nigra* Arnold). *Silvae Genet.* **43**: 20–30.

- SCHILLER, G. CONKLE, M. T. & GRUNWALD, C. 1986: Local differentiation among Mediterranean populations of Aleppo pine in their isoenzymes. *Silvae Genet.* 35: 11–19.
- SCHILLER, G. & KOROL, L. 1997: Electrophoretic analysis of diversity within *Cupressus sempervirens* L. growing in Israel. *Israel J. Plant Sci.* 45: 1–8.
- SCHILLER, G. & WAISEL, Y. 1989: Among-provenances variation in *Pinus halepensis* in Israel. *For. Ecol. Man*age., 28: 141–151.
- SCHMINCKE, H- U. 1976: The geology of the Canary Islands. *In:* Biogeography and ecology on the Canary Islands. Edited by G. Kunkle. Dr. W. Junk, b.v. Publish. The Hague. pp. 34–58.
- STRAUSSS, S. H. & DOERKSEN, A. H. 1990: Restriction fragment analysis of pine phylogeny. *Evolution* 44: 1081 -1096.
- SWOFFORD, L. D. & SELANDER, B. R. 1981: BIOSYS-1, A computer program for the analysis of allelic variation in genetics. User's manual. Department of Genetics and Development. University of Illinois at Urbana-Champaign. Urbana.
- TEAR, F. J. 1930: Government of Palestine, Department of Agriculture and Forests, Report for the Years 1927–1930.
- TEISSEIRE, H., FADY, B. & PICHOT, C. 1995: Allozyme variation in five French populations of Aleppo pine (*Pinus halepensis Mill.*). Forest Genetics 2: 225–236.
- WESTFALL, R. D. & CONKLE, M. T. 1992: Allozyme markers in breeding zone designation. New Forest 6: 279–309
- WRIGHT, S. 1965: The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**: 395–420.

						Populatior	1				
Locus	1	2	3	4	5	6	7	8	9	10	11
<i>Aap</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000
2	0.944	0.971	1.000	0.935	0.984	0.905	0.967	1.000	0.917	1.000	0.972
3	0.056	0.029	0.000	0.065	0.016	0.095	0.017	0.000	0.083	0.000	0.028
Aco	18	17	15	23	31	21	30	13	12	9	18
1	0.194	0.176	0.500	0.261	0.129	0.143	0.117	0.154	0.208	0.389	0.194
2	0.806	0.824	0.467	0.717	0.839	0.833	0.767	0.808	0.750	0.611	0.806
3	0.000	0.000	0.033	0.022	0.032	0.024	0.117	0.038	0.042	0.000	0.000
Acp	18	17	15	23	31	21	30	13	12	9	18
1	0.167	0.206	0.267	0.261	0.258	0.238	0.367	0.269	0.042	0.444	0.111
2	0.833	0.794	0.700	0.522	0.371	0.667	0.633	0.731	0.333	0.167	0.444
3	0.000	0.000	0.033	0.043	0.000	0.000	0.000	0.000	0.000	0.056	0.389
4	0.000	0.000	0.000	0.174	0.371	0.000	0.000	0.000	0.625	0.167	0.056
0	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.000	0.000	0.167	0.000
Adh-1	18	17	15	23	31	21	30	13	12	9	18
1	0.194	0.147	0.067	0.043	0.113	0.095	0.133	0.308	0.083	0.111	0.417
2	0.778	0.706	0.867	0.761	0.758	0.857	0.800	0.462	0.583	0.611	0.583
3	0.028	0.147	0.067	0.196	0.129	0.048	0.067	0.231	0.333	0.278	0.000
Adh2	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.000	0.971	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cat	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.032	0.024	0.000	0.000	0.000	0.000	0.000
2	1.000	1.000	1.000	1.000	0.968	0.976	1.000	1.000	1.000	1.000	1.000
<i>Est</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.056	0.059	0.067	0.065	0.048	0.071	0.083	0.000	0.000	0.000	0.028
2	0.111	0.000	0.000	0.043	0.065	0.048	0.033	0.000	0.000	0.222	0.000
3	0.722	0.912	0.800	0.826	0.774	0.881	0.850	1.000	0.667	0.778	0.833
4	0.111	0.029	0.133	0.065	0.113	0.000	0.033	0.000	0.333	0.000	0.139
Gdh	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056
2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.944
Got-1	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.972	1.000	0.967	0.978	0.984	1.000	0.983	1.000	0.958	1.000	1.000
3	0.028	0.000	0.033	0.022	0.016	0.000	0.017	0.000	0.042	0.000	0.000
Got-2	18	17	15	23	31	21	30	13	12	9	18
1	0.028	0.029	0.033	0.000	0.000	0.000	0.017	0.000	0.042	0.000	0.028
2	0.944	0.971	0.933	1.000	1.000	1.000	0.983	1.000	0.958	1.000	0.972
3	0.028	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

1						Populatior	1				
Locus	12	13	14	15	16	17	18	19	20	21	22
Aap	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000
2	0.900	0.944	0.976	0.983	0.982	1.000	1.000	1.000	0.964	1.000	1.000
3	0.100	0.056	0.024	0.017	0.018	0.000	0.000	0.000	0.018	0.000	0.000
Aco	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.024	0.000	0.089	0.034	0.071	0.074	0.054	0.033	0.143
2	1.000	1.000	0.976	0.983	0.911	0.948	0.905	0.926	0.946	0.833	0.786
3	0.000	0.000	0.000	0.017	0.000	0.017	0.024	0.000	0.000	0.133	0.071
Acp	10	27	21	30	28	29	21	27	28	30	7
1	0.300	0.241	0.310	0.283	0.286	0.241	0.190	0.259	0.125	0.233	0.357
2	0.350	0.759	0.690	0.717	0.679	0.759	0.738	0.741	0.875	0.750	0.643
3	0.100	0.000	0.000	0.000	0.036	0.000	0.071	0.000	0.000	0.000	0.000
4	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Adh-1	10	27	21	30	28	29	21	27	28	30	7
1	0.050	0.204	0.238	0.150	0.107	0.259	0.262	0.000	0.607	0.217	0.357
2	0.850	0.741	0.643	0.800	0.768	0.707	0.738	0.963	0.304	0.683	0.571
3	0.100	0.056	0.119	0.050	0.125	0.034	0.000	0.037	0.089	0.100	0.071
Adh-2	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.024	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000
2	1.000	1.000	0.976	1.000	1.000	1.000	0.952	1.000	1.000	1.000	1.000
Cat	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.037	0.000	0.033	0.000	0.000	0.000	0.352	0.000	0.000	0.000
2	1.000	0.963	1.000	0.967	1.000	1.000	1.000	0.648	1.000	1.000	1.000
<i>Est</i>	10	27	21	30	28	29	21	27	28	30	7
1	0.150	0.074	0.143	0.200	0.304	0.086	0.048	0.111	0.125	0.033	0.000
2	0.000	0.000	0.095	0.000	0.036	0.103	0.000	0.000	0.161	0.000	0.143
3	0.850	0.870	0.738	0.750	0.625	0.155	0.929	0.796	0.696	0.883	0.857
4	0.00	0.056	0.024	0.050	0.036	0.707	0.024	0.093	0.018	0.083	0.000
Gdh	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.037	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
2	1.000	0.963	1.000	1.000	1.000	1.000	0.976	1.000	1.000	1.000	1.000
Got-1	10	27	21	30	28	29	21	27	28	30	7
1	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
2	0.950	0.981	1.000	1.000	1.000	0.948	1.000	0.963	0.946	0.950	1.000
3	0.000	0.019	0.000	0.000	0.000	0.052	0.000	0.037	0.054	0.033	0.000
Got-2	10	27	21	30	28	29	21	27	28	30	7
1	0.100	0.019	0.048	0.050	0.018	0.000	0.000	0.000	0.000	0.000	0.143
2	0.900	0.981	0.833	0.950	0.929	1.000	1.000	1.000	1.000	1.000	0.857
3	0.000	0.000	0.119	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000

Leone						Populatior	1				
Locus	1	2	3	4	5	6	7	8	9	10	11
Got3	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.029	0.000	0.022	0.016	0.024	0.017	0.000	0.000	0.000	0.028
3	0.028	0.000	0.000	0.000	0.932	0.932	0.900	0.000	0.000	0.944	0.917
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-1	18	17	15	23	31	21	30	13	12	9	18
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-2	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.028
2	0.806	0.941	0.933	0.848	0.887	0.905	0.867	1.000	0.792	0.944	0.833
4	0.194	0.009	0.007	0.130	0.000	0.093	0.133	0.000	0.208	0.036	0.139
Mdh-3	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.029	0.000	0.000	0.000	0.000	0.033	0.038	0.000	0.000	0.083
2	0.028	0.088	0.067	0.022	0.048	0.000	0.033	0.000	0.000	0.111	0.028
5 4	0.000	0.000	0.000	0.000	0.016	1.000	0.000	0.000	0.042	0.000	0.056
0	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.055	0.000
Mdh-4	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.022	0.016	0.000	0.017	0.000	0.000	0.056	0.000
2 3	1.000 0.000	1.000 0.000	1.000 0.000	0.978 0.000	0.984 0.000	1.000 0.000	0.983 0.000	1.000 0.000	1.000 0.000	0.944 0.000	1.000 0.000
Mnr–1	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.022	0.016	0.000	0.017	0.077	0.000	0.000	0.000
2	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.944	1.000	0.967	0.913	0.984	1.000	0.917	0.885	0.958	1.000	0.944
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0	0.000	0.000	0.000	0.022	0.000	0.000	0.007	0.000	0.000	0.000	0.000
Mnr–2	18	17	15	23	31	21	30	13	12	9	18
1	0.333	0.441	0.367	0.435	0.258	0.310	0.483	0.154	0.125	0.389	0.333
2	0.639	0.559	0.633	0.565	0.710	0.643	0.517	0.846	0.875	0.611	0.667
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mnr–3	18	17	15	15	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.00
2 3	1.000 0.000	0.971 0.029	1.000 0.000	$1.000 \\ 0.000$	0.935 0.016	0.952 0.048	0.967 0.033	$1.000 \\ 0.000$	1.000 0.000	1.000 0.000	0.944 0.056
Mnr-5	18	17	15	15	31	21	30	13	12	9	18
1	0.389	0.324	0.433	0.609	0.355	0.429	0.467	0.269	0.417	0.389	0.694
2	0.611	0.676	0.567	0.391	0.645	0.571	0.533	0.731	0.583	0.611	0.306
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Lagua						Populatior	1				
Locus	12	13	14	15	16	17	18	19	20	21	22
Got-3	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.033	0.018	0.000	0.000	0.040	0.000	0.050	0.000
2	0.900	0.889	0.833	0.817	0.875	0.931	0.976	0.833	0.946	0.900	1.000
3	0.100	0.111	0.143	0.150	0.107	0.069	0.024	0.130	0.054	0.017	0.000
4	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000
<i>Lap-1</i>	10	27	21	30	28	29	21	27	28	30	7
1	0.850	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0	0.050	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Lap-2</i>	10	27	21	30	28	29	21	27	28	30	7
1	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.018	0.000	0.143
2	0.750	0.889	0.976	0.983	0.929	0.845	0.905	0.926	0.982	1.000	0.857
3	0.150	0.111	0.024	0.017	0.071	0.155	0.095	0.000	0.000	0.000	0.000
4	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Mdh–3</i>	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.119	0.117	0.143	0.034	0.000	0.093	0.036	0.033	0.000
2	0.150	0.056	0.048	0.017	0.000	0.034	0.095	0.037	0.143	0.083	0.000
3	0.150	0.056	0.048	0.000	0.125	0.000	0.048	0.019	0.071	0.117	0.000
4	0.700	0.889	0.786	0.867	0.732	0.914	0.833	0.852	0.750	0.767	1.000
0	0.000	0.000	0.000	0.000	0.000	0.017	0.024	0.000	0.000	0.000	0.000
<i>Mdh</i> 4	10	27	21	21	28	29	21	27	28	30	7
1	0.000	0.037	0.048	0.017	0.036	0.069	0.000	0.019	0.000	0.000	0.000
2	1.000	0.963	0.929	0.967	0.964	0.931	1.000	0.981	1.000	1.000	1.000
3	0.000	0.000	0.024	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Mnr-1</i> 1 2 3 4 5 0	10 0.000 0.850 0.000 0.150 0.000	27 0.000 0.944 0.019 0.037 0.000	21 0.000 0.048 0.881 0.000 0.071 0.000	21 0.000 0.000 0.883 0.050 0.067 0.000	28 0.000 0.964 0.000 0.036 0.000	29 0.017 0.000 0.862 0.034 0.086 0.000	21 0.000 0.095 0.905 0.000 0.000 0.000	27 0.130 0.019 0.852 0.000 0.000 0.000	28 0.054 0.000 0.911 0.000 0.018 0.018	30 0.000 0.917 0.000 0.000 0.000 0.083	7 0.000 0.000 1.000 0.000 0.000 0.000
<i>Mnr–2</i>	10	27	21	21	28	29	21	27	28	30	7
1	0.450	0.333	0.476	0.533	0.357	0.397	0.119	0.222	0.357	0.567	0.286
2	0.550	0.667	0.524	0.467	0.643	0.586	0.881	0.778	0.625	0.417	0.714
3	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.017	0.000
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000
<i>Mnr–3</i>	10	27	21	21	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.017	0.018	0.000	0.000	0.037	0.000	0.000	0.000
2	1.000	0.963	0.976	0.983	0.982	1.000	1.000	0.907	1.000	0.950	1.000
3	0.000	0.037	0.024	0.000	0.000	0.000	0.000	0.056	0.000	0.050	0.000
<i>Mnr–5</i>	10	27	21	21	28	29	21	27	28	30	7
1	0.600	0.481	0.619	0.817	0.554	0.776	0.524	0.630	0.714	0.700	0.857
2	0.400	0.519	0.381	0.183	0.446	0.224	0.476	0.370	0.250	0.300	0.143
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000

						Populatior	1				
Locus	1	2	3	4	5	6	7	8	9	10	11
Pep-1	18	17	15	23	31	21	30	13	12	9	18
1	1.000	1.000	1.000	0.978	1.000	1.000	0.967	1.000	1.000	1.000	1.000
2	.0.000	0.000	0.000	0.022	0.000	0.000	0.033	0.000	0.000	0.000	0.000
<i>Pep-2</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.972	0.941	1.000	1.000	0.855	0.976	0.867	1.000	0.792	1.000	0.889
3	0.028	0.059	0.000	0.000	0.145	0.024	0.133	0.000	0.208	0.000	0.111
<i>Pep-3</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.028
2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.958	1.000	0.972
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Pgd–1</i>	18	17	15	23	31	21	30	13	12	9	18
1	1.000	1.000	1.000	1.000	1.000	1.000	0.967	1.000	0.958	1.000	1.000
0	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.042	0.000	0.000
Pgd-2	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
2	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000
3	0.917	0.971	0.933	0.957	0.968	0.976	0.983	0.962	0.958	0.778	1.000
4	0.083	0.029	0.033	0.043	0.032	0.024	0.017	0.038	0.042	0.000	0.000
<i>Pgd–3</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.111	0.176	0.300	0.000	0.016	0.000	0.017	0.000	0.000	0.111	0.028
2	0.639	0.647	0.633	0.804	0.887	1.000	0.817	1.000	0.625	0.889	0.972
3	0.250	0.059	0.067	0.196	0.097	0.000	0.167	0.000	0.250	0.000	0.000
4	0.000	0.118	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000
<i>Skdh–1</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.056	0.029	0.100	0.152	0.000	0.048	0.083	0.038	0.000	0.000	0.028
2	0.944	0.971	0.867	0.739	1.000	0.952	0.850	0.962	0.958	0.889	0.917
3	0.000	0.000	0.033	0.109	0.000	0.000	0.067	0.000	0.042	0.111	0.056
<i>Skdh–2</i>	18	17	15	23	31	21	30	13	12	9	18
1	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.056	0.000
2	1.000	1.000	1.000	1.000	1.000	0.952	1.000	1.000	1.000	0.944	1.000
3	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
Sod	18	17	15	23	31	21	30	13	12	9	18
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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T						Population	1				
Locus	12	13	14	15	16	17	18	19	20	21	22
Pep-1	10	27	21	30	28	29	21	27	28	30	7
1	1.000	1.000	1.000	1.000	1.000	1.000	0.976	1.000	1.000	1.000	1.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000
Pep-2	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000
2	0.950	0.981	0.976	0.983	0.893	0.879	0.952	1.000	1.000	0.967	1.000
3	0.050	0.019	0.024	0.017	0.107	0.121	0.048	0.000	0.000	0.000	0.000
Pep-3	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.037	0.143	0.000	0.000	0.000	0.000	0.000	0.036	0.033	0.000
2	0.950	0.963	0.857	1.000	1.000	1.000	1.000	1.000	0.964	0.967	1.000
3	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgd-1	10	27	21	30	28	29	21	27	28	30	7
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.857
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.143
Ped-2	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.017	0.000	0.000	0.095	0.000	0.018	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.019	0.000	0.000	0.000
3	1.000	1.000	0.905	0.983	0.946	0.914	0.833	0.889	0.839	0.817	1.000
4	0.000	0.000	0.095	0.000	0.054	0.086	0.024	0.093	0.143	0.183	0.000
Pgd-3	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.111	0.024	0.000	0.196	0.103	0.048	0.000	0.143	0.267	0.000
2	1.000	0.778	0.976	1.000	0.732	0.897	0.810	0.685	0.839	0.700	1.000
3	0.000	0.111	0.000	0.000	0.071	0.000	0.024	0.315	0.000	0.017	0.000
	0.000	0.000	0.000	0.000	0.000	0.000	0.119	0.000	0.017	0.000	0.000
Skdh-1	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.352	0.000	0.033	0.000
2	1.000	0.963	0.976	0.967	1.000	0.966	1.000	0.648	1.000	0.967	1.000
3	0.000	0.000	0.024	0.033	0.000	0.034	0.000	0.000	0.000	0.000	0.000
Skdh-2	10	27	21	30	28	29	21	27	28	30	7
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.000	1.000	0.976	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
3	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sod	10	27	21	30	28	29	21	27	28	30	7
1	1.000	0.963	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 1. Allelic frequencies of 28 polymorphic loci in 22 populations of Pinus canariensis (continued).



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Appendix 2. Migration rates of alleles in the different enzyme systems. * signals the most common allele in each locus.