Electrophoretic and quantitative variation in chestnut (*Castanea sativa* Mill.) in Hellenic populations in old-growth natural and coppice stands

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Abstract
Chestnut cultivation is economically of interest in Greece for timber and nut production. However, no information exists on the genetic variability of Hellenic populations. In each of two geographically distant areas, winter buds and seeds were sampled in one natural old-growth and one coppice population of chestnuts (in total four populations). Eight enzyme systems were studied. Genetic variability within and between populations was found to be high and can be regarded as the highest reported among natural European populations. The comparison between old-growth natural and coppice populations showed no statistically significant differences in heterozygosity. Seven quantitative parameters were also recorded. Analysis of variance indicated significant differences between the populations studied. The high diversity observed in genetic and quantitative variation is very important for both the development of breeding applications and the conservation of the species' genetic resources.

Keywords: *Castanea,* Greece, isoenzymes, seed morphology, genetic variation

1 Introduction

Sweet chestnut (*Castanea sativa* Mill.) is one of the more widespread broad-leaved tree species in southern Europe and in Mediterranean areas. It is widely cultivated for timber and nut production. In rural Greece, chestnut cultivation is very important and represents an integral part of the economy in many areas (DIAMANDIS and PERLEROU 1996). This multi-purpose species requires sound management and appropriate decisions to ensure genetic improvement. When selecting gene conservation strategies and practices, at both national and European levels, the magnitude and structure of genetic variation in natural populations must be known. Up until now there has been no study of the genetic variation of Hellenic populations. In this paper, an initial assessment of population variability, in terms of genetic (isoenzymatic) and quantitative (morphometric) variation, is presented. Morphological characters usually exhibit a greater degree of differentiation between populations than isoenzymes (HAMRICK 1983) and it has been indicated that different evolutionary forces may be acting upon these different sets of characters (MITTON 1983). Besides investigating two different types of variation, this assessment capitalises on the local coexistence of two different types of domestication, old-growth natural stands and coppice stands. This means it is possible to evaluate their relative levels of variability. This study can also be seen as an initial investigation of the possible impact of anthropogenic management practices on the diversity parameters of forest populations.

2 Materials and methods

Four populations from two geographically distant (100 km apart) areas, Mt. Hortiatis and Mt. Paikon, were sampled with regard to winter buds and seeds. Winter buds from a total of 26 trees per population were sampled from each of the two domestication levels at Mt. Hortiatis (old-growth natural and coppice) and the corresponding two levels at Mt. Paikon. They were then used for enzyme extraction and horizontal starch gel electrophoresis (ARAVANOPOULOS *et al.* 1994, 2000). Eight enzyme systems were studied (Table 1). Enzyme electrophoresis was repeated twice with independent samples over a period of two years, and replicable results were obtained. For estimating genetic parameters, the BIOSYS-1 (SWOFFORD and SELANDER 1981) software was used.

Twenty seeds from 28 different trees per population (which included the 26 trees used in the isoemzymic analysis), i.e a total of 560 seeds, were collected for the study of the two natural populations. 20 seeds were taken from eight different trees (a subsample of the 28 trees sample) per population i.e. a total of 160 seeds, for the comparison of the old-growth natural and coppice populations of Mt. Hortiatis. Six morphological parameters of the seed (length, width, thickness, distance from base to widest point, hylum length, hylum width), as well as seed weight, were recorded to the nearest mm and mg in all populations except the coppice population of Mt. Paikon. For the latter, the sample sizes available prevented sound statistical analysis. Descriptive statistics were estimated for all the populations tested, with respect to all seed traits. Analysis of variance was employed to investigate the levels of within and between population variation of: (a) old-growth natural populations of Mt. Hortiatis and Mt. Paikon, and (b) old-growth natural and coppice populations of Mt. Hortiatis. The following linear model was used in the analysis:

$$Y_{ijk} = \square + P_i + G(P)_{j(i)} + \varepsilon_{ijk}$$

where Y_{ijk} is the value of the quantitative parameter in the k*th* seed of the j*th* genotype (tree) in the i*th* population; $\square$ is the overall mean for the respective morphological parameter; P_i is the effect of the i^{th} population, $G(P)_{j(i)}$ is the effect of the j*th* genotype within the i*th* population and ε_{ijk} is the error term (deviation of the k*th* seed of the j*th* genotype in the i*th* population). Effects were considered random. The SAS software package was used (SAS 1985).

Table 1. Enzyme systems investigated, with their Enzyme Commission (E.C.) number, abbreviation, and numbers of scored loci, variable loci and alleles in *Castanea sativa.*

Enzyme	E.C. No.	Abbreviation	Scored Loci	Variable Loci	Alleles
Alcohol dehydrogenase	1.1.1.1.	ADH	1	1	2
Diaphorase	1.6.4.3.	DIA	2	1	3
Esterase	3.1.1.1.	EST	1	1	3
Glutamate oxaloacetate transaminase	2.6.1.1.	GOT	1	1	2
Isocitrate dehydrogenase	1.1.1.42.	IDH	1	1	2
6-Phosphogluconate dehydrogenase	1.1.1.44.	6-PGD	1	1	2
Phosphoglucose isomerase	5.3.1.9.	PGI	2	2	5
Shikimate dehydrogenase	1.1.1.25.	SDH	1	1	2
Totals		8	10	9	21

3 Results

A total of 10 loci (nine polymorphic) and 21 alleles were revealed out of the eight enzyme systems studied (Table 1). All enzyme systems were polymorphic and a total of nine polymorphic loci were identified. The results of the genetic diversity parameters are given in Table 2. The average number of alleles per locus was 1.925. The percentage of polymorphic loci ranged from 60% to 80% with an average value of 67.5%. Observed heterozygosity was generally high with a range of 0.215 to 0.333 and a mean value of 0.278. Gene diversity was also notable with an average value of 0.285 and a range of 0.211 to 0.351.

The descriptive statistics of the seed quantitative traits from the Mt. Paikon and Mt. Hortiatis natural populations are presented in Table 3. For all the seed characters tested, higher mean values were scored for Mt. Paikon natural population. The Mt. Hortiatis natural population can be considered as more variable since the coefficient of variation (CV) values estimated for all seed traits was higher. The results obtained from the analysis of variance of all seed characters are given in Table 4. Significant differences were revealed among and within populations. Table 5 depicts the descriptive statistics of the seven seed traits for the Mt. Hortiatis old-growth natural and coppice populations. In this case, the mean value for the coppice population were higher and more variable than those for the natural one, as indicated by the respective CV values. The associated analysis of variance indicated that the two population types differed for both levels tested (Table 6). The most variable seed character was seed weight, with CV values ranging from 37% to 69%.

Table 2. Parameters of genetic diversity in *Castanea sativa* (standard errors in parentheses). [1] Average number of alleles per locus; [2] Percent polymorphic loci (where the frequency of the most common allele does not exceed 0.95); [3] Observed heterozygosity; [4] Gene diversity (expected heterozygosity, unbiased estimate; NEI, 1978).

Population	A[1]	P[2]	H[3]	G[4]
Mt. Hortiatis Natural	1.900 (0.200)	80.0	0.311 (0.061)	0.324 (0.062)
Mt. Hortiatis Coppice	2.000 (0.200)	70.0	0.333 (0.076)	0.351 (0.074)
Mt. Paikon Natural	1.900 (0.200)	60.0	0.228 (0.070)	0.254 (0.064)
Mt. Paikon Coppice	1.900 (0.200)	60.0	0.215 (0.073)	0.211 (0.062)
Mean	1.925 (0.050)	67.5	0.272 (0.058)	0.285 (0.064)

Table 3. Characters of natural populations of *Castanea sativa* on Mt. Paikon and Mt. Hortiatis: means, standard deviations and coefficients of variation (CV) of seven seed characters (length – SL, distance from base to widest point – BW width – SW, thickness – ST, hylum length – HL, hylum width – HW, seed weight – WGT).

Population		SL	BW	SW	ST	HL	HW	WGT
Mt. Paikon	Mean	2.496	0.849	2.388	1.488	1.453	0.757	6.075
	St. deviation	0.324	0.187	0.348	0.273	0.276	0.151	2.269
	CV	12.981	22.026	14.573	18.347	18.995	19.947	37.350
Mt. Hortiatis	Mean	1.911	0.661	1.886	1.082	1.295	0.606	2.988
	St. deviation	0.300	0.159	0.387	0.264	0.286	0.151	1.567
	CV	15.699	24.054	20.520	24.399	22.085	24.917	52.443

Table 4. Seed characters of natural populations of *Castanea sativa* on Mt. Paikon and Mt. Hortiatis: (length – SL, width – SW, thickness – ST, distance from base to widest point – BW, hylum length – HL, hylum width – HW, seed weight – WGT). *significant for p=0.01.

		Mean Squares						
Source	df	SL	SW	ST	BW	HL	HW	WGT
Population	1	95.876*	70.667*	46.134*	9.991*	7.033*	6.374*	2667.693*
Tree/Pop.	54	1.291*	1.655*	0.684*	0.274*	0.815*	0.255*	48.144*
Error	1064	0.037	0.058	0.041	0.018	0.041	0.011	1.554
Total	1119							
R^2		0.808	0.721	0.656	0.567	0.537	0.636	0.761

Table 5. Characters of natural and coppice populations of *Castanea sativa* on Mt. Hortiatis: means, standard deviations and coefficients of variation (CV) of seven seed characters (length – SL, distance from base to widest point – BW width – SW, thickness – ST, hylum length – HL, hylum width – HW, seed weight – WGT).

Population		SL	BW	SW	ST	HL	HW	WGT
Natural	Mean	1.721	0.602	1.686	1.007	1.169	0.577	2.439
	St. deviation	0.389	0.162	0.450	0.327	0.301	0.147	1.574
	CV	22.603	26.910	26.690	32.473	25.748	25.477	64.535
Coppice	Mean	2.103	0.823	2.010	1.137	1.384	0.650	3.694
	St. deviation	0.566	0.308	0.665	0.401	0.488	0.223	2.574
	CV	26.914	37.424	33.085	35.268	35.260	34.308	69.681

Table 6. Analysis of variance of seven seed characters of natural and coppice populations of *Castanea sativa* (length – SL, width – SW, thickness – ST, distance from base to widest point – BW, hylum length – HL, hylum width – HW, seed weight – WGT), from the Mt. Hortiatis natural and coppice populations of *Castanea sativa.**significant for p = 0.01.

		Mean Squares						
Source	df	SL	SW	ST	BW	HL	HW	WGT
Population	1	11.680*	8.346*	1.339*	3.891*	3.668*	0.427*	125.713*
Tree/Pop.	14	4.718*	6.338*	2.117*	1.014*	2.969*	0.571*	82.477*
Error	304	0.029	0.045	0.042	0.017	0.035	0.011	0.943
Total	319							
R^2		0.898	0.707	0.877	0.704	0.809	0.721	0.818

4 Discussion

The numbers of enzyme systems and loci studied are similar to those of other studies on *Castanea sativa,* which vary from three enzyme systems and loci (FRASCARIA *et al.* 1993) to 13 enzyme systems and 16 loci (VILLANI *et al.* 1991b). The genetic diversities were similar in old growth natural and coppice populations at each locality with no statistically significant differences in the heterozygosity of the populations of *C. sativa* (t-tests; results not shown). On the other hand, the results for the two localities did differ significantly, and the Mt. Hortiatis natural and coppice populations showed greater heterozygosity and gene diversity than the corresponding Mt. Paikon populations (t-tests; results not shown).

In general, heterozygosity values were within the range reported for populations of *C. sativa* in Asia Minor, but higher than those reported for Italian populations (VILLANI *et al.* 1991a, 1991b, 1992). Gene diversity was higher than that of Italian, French and Turkish populations (VILLANI *et al.* 1991a, MACHON *et al.* 1996). The genetic variability within and between populations seems to be very high, and is probably the highest reported for natural European populations. The genetic parameters studied indicate the absence of significant differences between the old-growth and respective coppice stands in a particular area.

The mean values of the Mt. Paikon natural population seed characters were higher than those for the corresponding characters from Mt. Hortiatis. Climatic conditions for chestnut growth are more favourable in the former area, which may explain the results observed. The variability in the Mt. Hortiatis populations was found to be higher. In this area, trees grow under considerable drought stress, which may contribute to the wider range of values

observed, or the population may possess higher inherent variability. Analysis of variance also showed significant differences between and within the Mt. Hortiatis and Mt. Paikon natural populations for all seed characters tested. VILLANI *et al.* (1992) also reported significant differences among Asia Minor *C. sativa* populations with respect to seed morphological characters. A comparison of the results for the genetic loci and the quantitative data shows similarties between the two types of variation in the *C. sativa* Hellenic populations studied. LEWONTIN (1984) pointed out that it is statistically much more difficult to detect gene frequency differences between populations than between means of metric characters when the two sets of data are equally differentiated. Nevertheless, in the present case, it seems that population differences are large enough for such variability to be detected.

The mean values for the seed morphological parameters in the Mt. Hortiatis coppice population were higher than the corresponding ones fot the natural population, while their coefficient of variation values were also higher. Seed weight generally presented high CV values, which could be attributed to the fact that it is a more complex quantitative trait that is affected by other seed morphometric traits. A comparison of the old-growth natural and coppice stands also indicated the existence of differences for the two population types with respect to the parameters studied. This result is not in agreement with the findings of the gene loci analysis, where the genetic diversity differences were not significant. It is difficult to interpret this finding biologically. The analysis of the isoenzyme data was based on a much larger sample than the analysis of the morphometric data. A possible sampling effect may have contributed to the results observed. Higher sample sizes and repeated measurements over different years may be needed before a conclusion can be drawn.

In general, results indicated considerable quantitative variation in the populations studied and the existence of population differences. The presence of high isoenzymatic and quantitative variation indicates that there is considerable potential in Greece for breeding chestnuts in order to improve the most important traits. Results also suggest that the levels of genetic diversity in natural and coppice populations within a particular geographic locality do not seem to differ significantly. This implies that any anthropogenic influences induced by different management techniques did not have any adverse effect on the *C. sativa* populations studied.

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