Genetic Variation in Juvenile Height and Biomass of Open-pollinated Families of six *Castanea sativa* MILL. Populations in a 2 x 2 Factorial Temperature x Watering Experiment

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Abstract

The objective of our study was to assess the amount of within- and among-population variation of traits of adaptive significance such as juvenile height growth, stem, leaf, root, and total dry weight of sweet chestnut (*Castanea sativa* MILL.) and to evaluate how genetic variation is affected by different combinations of temperature and water availability. Forty-eight open-pollinated families from a stratified sample (temperature/precipitation) of 6 natural/naturalized populations from Spain, Italy, and Greece were grown for one growth period in a factorial 2 x 2 temperature –(*25 and 32°C*) and watering –(*well watered or periodic drought*) experiment in growth chambers. Strongly significant effects of the water and temperature treatments, and *temperature x water* interaction were found for most growth traits. Significant population effects were found for all traits studied. The significant *population x temperature x watering* interaction indicated presence of some specific adaptedness. The family effects were strongly significant for all traits except for the root/stem weight ratio. The heritabilities and CV_A for growth traits were generally large (0.42–0.89 and 16.2–56.6% respectively). The highest estimates were obtained in the 25 °C temperature and drought treatment. The variance components for the 3-way interaction were much smaller than the family variance components. Depending on the trait, 7–13 families contributed significantly to the *g x e* interaction with generally low estimates of the ecovalence values, <6.6%. All populations studied demonstrated a large variation in CV_A of height growth and biomass traits with diverse patterns of its change across the treatments. The large additive variance for height growth and biomass traits found within the six studied populations of *Castanea sativa* suggests good prospects for adaptation during the juvenile stage to future climates.

Key words: Sweet chestnut, temperature, drought, genetic variation, adaptive traits

Introduction

Forest tree species have faced long-term changes in environmental conditions during the last 13,000 years by migration and/or adaptation to the most suitable habitats (HUNTLEY and BIRKES, 1983). However, the ability of forest tree species to cope with the quick global climate warming foreseen in the next decades is unclear. One projection is that more extremes with respect to temperature and precipitation will occur (KATTENBERG et al., 1996). With periods of drought, high and low temperatures becoming more frequent, the environmental stress on trees will increase. Moreover, increased temperature and humidity are expected to favour epidemic spread of pathogens and parasites in temperate regions. The projections for future climatic conditions must be considered when gene conservation of a tree species is designed. Of greatest significance for the long-term gene conservation is the presence of sufficient additive variance in traits of adaptive significance (RITLAND, 1996; ERIKSSON, 2000).

Over many centuries, man has influenced the European chestnut populations through propagation and transplantation of plant material, intensive cultivation of grafted (clonal) trees, silvicultural practices and fragmentation of populations owing to changes in land use. Severe fungal attacks of *Cryphonectria parasitica* during the last 5–6 decades combined with the increasing spread of *Phytophthora sp.* all over the natural range, have also led to a reduction of population size and to depauperation of extant chestnut genetic stocks in many European countries.

Small populations on highly fragmented habitats, geographically or ecologically isolated from the main gene pools, are expected to lose genetic variation by random genetic drift (GILPIN, 1991; YOUNG et al., 1996). Genetically depleted populations are less likely to cope with environmental stress under changing environmental conditions and may be more prone to inbreeding depression, with loss of productive performance (ELLSTRAND, 1992). Gene flow is an important homogenizing factor counterbalancing divergence and loss of genetic variability by drift (cf SLATKIN, 1987). The variability of gene pools mainly depends on an adequate level of gene flow among stands and the maintenance of a substantial population size (CARSON, 1990). The large cultivation of grafted varieties over the distribution area of chestnut may have caused the introgression of foreign germ plasm into local gene pools via gene flow and may have disrupted local adaptedness.

The wide distribution of chestnut throughout Southern Europe exposed this species to a variety of environmental conditions. Its expansion occurred under both natural and artificial selective forces in a changing environment, which probably caused a population differentiation in adaptive traits. Such a capacity for dynamic colonisation in a species has been attributed to a large adaptability of the species (cf. ERIKSSON et al., 1993).

There is considerable knowledge on genetic differentiation of *Castanea sativa* as regards isoenzyme markers (eg. VILLANI and PIGLIUCCI, 1991). A large genetic variability has been detected, especially in the Ponto-Caucasian region (VILLANI et al., 1999). However it is generally accepted that markers are neutral and do not reflect adaptive variation (eg. KARHU et al., 1996). In view of future global change there is a need to understand the genetic basis of traits which are likely to have a high adaptive value, such as drought tolerance, avoidance of exposure to late spring frosts or early autumn frosts (phenological traits), and tolerance to disease. Only a few studies have been carried out on this subject (LAUTERI et al., 1999) and they have been restricted to populations originating from limited regions of the whole chestnut distribution area. High mortality and

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small differentiation among populations were found in the degree of susceptibility to artificial *Cryponectria parasitica* inoculation (COLINAS et al., 1999; XENOPOULOS et al., 1999).

Ecophysiological studies highlighted differential mechanisms of response to water stress depending on the site conditions at the origins (LAUTERI et al., 1997a, 1997b). A higher capacity of photosynthesis and transpiration in seedlings from arid sites than in those from mesic sites was reported (LAUTERI et al., 1999). Variability in bud burst, a phenological trait related to avoidance of late spring frost, was detected among chestnut provenances belonging to these contrasting climatic environments (LAUTERI et al., 1997a). These results suggest that there is genetic variation in drought tolerance and phenology which thus calls for a study of the genetic variation in these traits in material from the whole distribution area of the species.

The aim of our study was to assess the genetic variation of traits of adaptive significance such as drought tolerance, juvenile growth, and biomass distribution of open-pollinated families from a stratified sample (temperature/precipitation) of populations from the European distribution area of the species. Partitioning of additive variance among and within populations as well as estimates of stability will be presented. The data obtained will be discussed with respect to the gene conservation of *Castanea sativa*.

Material and Methods

Material

The plant material used consisted of 48 open-pollinated families of *Castanea sativa* from 6 natural/naturalised populations growing on sites of ecological extremes in Greece, Italy and Spain (*Table 1*). The term 'naturalized' means that the populations originated from natural regeneration. Each population was represented by 8 open-pollinated families.

Table 1. – *Castanea sativa* populations and some climatic characteristics of their sites.

EU code	Population name	Village name	Region	Country	Longitude	Latitude	Annual precipitation, mm	Annual mean temp., °C
SP7	**Málaga**	Gaucín	Málaga	Spain	5°18'21"W	36°32'10" N	1214	13.7
SP11	**A Coruña**	Cambre	A Coruña	Spain	8°22'25" W	43°17'15" N	1148	12.8
I6	**Sicilia I**	Petralia Sottana	Sicilia I	Italy	14°05'20" E	37°49'22"N	770	13.6
I25	**Pellice**	Villar Pellice	Piemonte I	Italy	7°09'00" E	44°49'00"N	1216	10.3
GR1	**Paiko**	Griva	Northern Mac.	Greece	22°22'18" E	40°57'42"N	558	12.0
GR5	**Hortiatis**	Hortiatis	Central Mac.	Greece	23°09'52" E	40°22'43"N	461	15.7

The progenies were grown in climatic chambers. The experimental design was randomized single-tree plots with one seedling per family in each of the 20 replications per treatment. Seed (nuts) were sown in single plastic containers (9 x 9 x 20 cm, volume 140 cm^3). Containers were filled with pumice (grain size 0.1–1.0 mm). Containers were put into 50 x 45 x 15 cm boxes and placed on trucks with 20 containers per box/truck, with an average spacing between seedlings of 10 cm. Two nuts were sown in each container. After 4 weeks non-germinated nuts or one of two germinated nuts were removed.

Treatments

At the germination phase all seedlings were grown for 5 weeks at 25 °C temperature and normal watering before the treatment started. After that progenies from all 6 populations were grown for 9 weeks under two temperature regimes – 25 and 32°C in combination with 2 irrigation (watering) regimes – watering to full field capacity (= well watered) and periodic drought. The factorial combination of temperature and irrigation resulted in four treatments:

Temperature 25° well watered = T25W;

Temperature 25° periodic drought = T25D;

Temperature 32° well watered = T32W;

Temperature 32° periodic drought = T32D.

The containers with seedlings in the T25W treatment were watered to full field capacity every day for the 4 weeks. During the last 5 weeks, the seedlings were watered every second day. In the T32W treatment the seedlings were watered every day for the whole 9 weeks growth period. In the T25D treatment the watering was done every 4th day and in the T32D treatment it was done every 3rd day. The drought stress was not intended to kill any seedlings but to reduce their growth compared to the well-watered seedlings in the same temperature treatment.

Every watering was done using balanced nutrient solution containing 102 mg/l N (40 mg ammonium + 62 mg nitrate), 20 mg/l P, 86 mg/l K, 6 mg/l Ca, 8 mg/l Mg, 8 mg/l S, and microelements according to INGESTAD and LUND (1986). The plants were watered to saturation supplying solution from below the containers by filling the large box with solution up to 10 cm level for 30 minutes, then allowing all residual water to drain away.

The seedlings were grown in light from daylight lamps providing an irradiance of about 400 μ $mol \cdot m^{-2} \cdot s^{-1}$ in the 400–700 nm spectrum. The photoperiodic regime, 16 h daylight and 8 h darkness.

Assessments

The height of the seedlings was recorded every week. The height of the seedlings (H) was measured from the edge of the plastic container to the top of the plant. After one growth period (14 weeks), the seedlings were harvested. Leaves and roots were separated from stem, put in paper bags and dried to constant weight in an oven for 48 hours at 45 °C and then for 24 hours at 80°C. Dry weights of stem (SDW), leaves (LDW), and root (RDW) were recorded separately. Total dry weight (TDW), root/stem dry weight ratio (RSDW), and root/leaf dry weight ratio (RLDW) were derived from the assessed individual weights. The branching was scored 1 to 4 (1 – no branches, 2 – one-two branches, 3 – more than two branches, 4 – more than two branches with secondary branches).

Statistical analysis

In order to diminish the influence of significant differences in germination date on individual, family, and population level and suppression of growth on patterns of variation in final growth and biomass traits, several covariates and methods of culling of outliers were tested:

Covariates:

1. date of germination: a) date of first registration of height, b) extrapolation backwards on the growth curves, which in turn were obtained from the weekly recording of height;
2. nut weight;
3. heights at 25 and 35 days after sowing.

Culling of outliers:

1. final height was less than $\bar{x} - 2\sigma$;
2. limited growth during the second half of the growth period (less than 20 % from growth during the first half of the growth period);

3. limited growth of small seedlings (< $\bar{x}$ – 1σ) during the second half of the growth period.

In the ANOVA's reported in this paper we have selected to use as covariate (as fixed effect in the model of MIXED procedure) the interpolated day of germination (1b option). Additionally to covariates the combination of methods 1+3 for culling of outliers was applied. Most of the outliers removed by method 1 were seedlings of very late germination, while method 3 removed the seedlings that have slowed down the growth because of suppression. The reasons for the selection of covariate and culling are given in the Discussion.

Variance analysis was done using MIXED procedure in the SAS Software (SAS Institute Inc. SAS/STAT® software. Release 6.12). Mixed model equations (MME) and the restricted maximum likelihood (REML) method were used for computing variance components.

The following linear models were used for joint analyses of the four treatments together and for separate analyses of individual treatments:

1) Joint:

$$y_{ijklmn}=\mu+ag_n+b_i(tw)_{jk}+tw_{jk}+t_j+w_k+f_l(p)_m+p_m+ftw_{ljk}+ptw_{mjk}+\varepsilon_{ijklm}$$

2) Separate:

$$y_{ilmn}=\mu+ag_n+b_i+f_l(p)_m+p_m+\varepsilon_{ilm}$$

where y_{ijklm} and y_{ilm} – values of single observation, μ – grand mean, a – constant, g_n – fixed effect of germination date for individual n, t_j – fixed effect of temperature regime j, w_k – fixed effect of water regime k, tw_{jk} – fixed effect of interaction between temperature regime j and water regime k, $b_i(tw)_{jk}$ – fixed effect of block i within treatment jk, b_i – fixed effect of block i, $f_l(p)_m$ – random effect of family l within population m, p_m – fixed effect of population m, ftw_{lkj} – random effect of interaction between family l, water regime k and temperature regime j, ptw_{mjk} – fixed effect of interaction between population m, water regime k and temperature regime j, e_{ijklm} and e_{ilm} – random error terms.

Genetic parameter estimates

Genetic parameters were derived from model 2 separately for each treatment.

The families were considered as half-sibs and genetic parameters were interpreted as:

Additive genetic variance: $\sigma^2_A = 4\sigma^2_f$

Environmental variance: $\sigma^2_E = \sigma^2_e - 3\sigma^2_f$

Additive genetic coefficients of variation: $CV_A = \frac{\sqrt{4 \cdot \sigma^2_f}}{\overline{X}} \cdot 100$

Individual tree heritabilities: $h^2_i = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_A}$

Genetic correlations: $r_A = \frac{\sigma_{A_1A_2}}{\sqrt{\sigma^2_{A_1} x\ \sigma^2_{A_2}}}$

where σ^2_f – family variance component, σ^2_e – error variance component, $\overline{X}$ – he phenotypic mean of the trait, $\sigma_{A_1A_2}$ – covariance between trait 1 and 2, $\sigma^2_{A_1}$ and $\sigma^2_{A_2}$ – additive genetic variance of trait 1 and 2 respectively.

Standard errors of individual heritabilities were calculated as described for unbalanced designs in BECKER (1984).

Genetic correlations between the same trait assessed in different treatments were calculated to evaluate the contribution of each pair of treatments to total *genotype x environment* interaction. Genetic correlation coefficients were calculated at individual observation level and using the procedure AI-REML of ASReml software (GILMOUR et al., 2000a) based on mixed linear models. Block and population effects were set as fixed. Standard errors of the estimates were obtained by Taylor series approximation (GILMOUR et al., 2000b).

Stability analysis of populations and families

To estimate the contribution of each family to the *family x treatment* interaction variances, the ecovalence value (WRICKE, 1962) of families by populations was calculated on the individual observation level, using solutions to the mixed linear model (BLUP) for individual families within each treatment (Option: Solution, SAS procedure MIXED). The ecovalence value as a measure of interaction variance for each family was expressed in percent of the total interaction variance. This type of analysis was done for traits where *family x treatment* interaction was significant. The stability variances were computed and the significance of the ecovalences was tested using the method developed by SHUKLA (1972). In calculating ecovalences to better fulfill the assumptions behind the linear model and thus reduce the scale affects of different treatments in joint analysis, the data were transformed to equal additive genetic variance using the method of DANELL (1988). The method is further described by ERICSSON (1994). For each trait and treatment, the assessed values for each seedling were multiplied by a scaling factor which for the ith treatment was $k_i = \sigma_{\bar{A}}/\sigma_{Ai}$ where $\sigma_{\bar{A}}$ – mean additive genetic standard deviation over all 4 treatments, σ_{Ai} – additive genetic standard deviation for the ith treatment.

The contribution of each pair of treatments to total *genotype x environment* interaction was evaluated using genetic correlations between the same trait assessed in pairs of different treatments.

To estimate the contribution of each population to the *population x treatment* interaction variances, the ecovalence value of populations was computed, using the Lsmeans estimates of the mixed linear model (Option: Lsmeans, SAS procedure MIXED) for individual populations within each treatment. Individual observations were used. The population effect was considered as fixed. To reduce the scale affects of different treatments in joint analysis, the data were transformed to equal population additive genetic variance using the method described above substituting additive genetic standard deviations with the corresponding for populations.

Results

Treatment effects

As seen from *table 2* there were strongly significant effects of the water and temperature treatments for most traits. However, the coefficients of additive variance, CV_As, did not differ much between the two watering treatments while they differed between the two temperature treatments (*Table 3*). Also the effect *temperature x water* treatment was significant for most traits while the 3-way interaction was strongly significant for 2 traits only. The root/stem ratios were higher in the two drought treatments than in the corresponding well-watered treatments. The lowest root/stem ratio, 0.46, was noted for population mount Paiko in the T32W treatment while the highest ratio, 1.00 was noted for the same population in the T25D treatment.

Among-family variation

As seen from *table 2* the family effects were strongly significant for most traits. As seen from *figure 1* there is a large family variation in most populations in all four treatments. The heritabilities and CV_A were generally large (*Table 4*), the only exception to this being the heritabilities for root/stem ratios. There is a large variation in CV_A of the six populations for the total dry matter (*Fig. 2*). The mount Paiko Greek population

was extreme, almost without variation in the two T32 treatments. Of particular importance is the absence of a significant family effect for the root/stem ratio (*Table 2*).

Table 2. – Variance components for random effects as percent of the total random variation, and significance of all effects. σ^2_f, σ^2_{ftw} are the variance components for family, and family x water x temperature interaction, respectively. *p, t, w, tw, ptw* are the population, temperature, water, temperature x water, and population x temperature x water interaction fixed effects, respectively. Level of significance is denoted by: *–0.05>P>0.01, ** –0.01> P>0.001, *** –P<0.001. Results from joint mixed linear model analysis of variance of different traits of *Castanea sativa* open pollinated families over four treatments.

Trait	Variance components of random effects, %		Significance of fixed effects				
	σ^2_f	σ^2_{ftw}	*p*	*t*	*w*	*tw*	*ptw*
Height (H)	14.9***	3.3**	***	***	***	***	**
Stem dry weight (SDW)	11.7***	4.4***	***	***	***	***	*
Leaf dry weight (LDW)	13.4***	1.7	***	***	***	***	
Root dry weight (RDW)	11.9***	1.3	***	*		***	*
Total dry weight (TDW)	13.3***	2.5*	***	***	***	***	*
Root/stem ratio (RSDW)	1.5	1.5	***	***	***		
Root/leaf ratio (RSDW)	1.2	0.9	**	***	*		
Branchiness (BRAN)	4.2**	1.0	***	***	*	***	***

Among-population variation

From *table 2* it is seen that there was a strongly significant population variation in all traits. From *figure 3* it is seen that population Coruna, Spain, deviated positively while the population Hortiatis, Greece, deviated in the opposite direction. Of particular interest is that there was a strongly significant population variation in the root/stem ratio. The pairs of populations within each of the countries demonstrated rather pronounced differences in total dry weight and pattern of change in growth across the different treatments (*Fig. 3*). Except for branching the CV_As were larger in the populations from the dry sites (*Table 3*). The pattern of CV_As with respect to country was not consistent among traits.

Interactions

Three-way interaction *population x temperature x watering* was significant for most of traits (*Table 2*). The interactions *population x temperature* and *population x watering* treatment were not significant for any trait (not shown) when 3-way interaction was included into the joint model (1).

As seen from *table 2* the 3-way family interaction variance component was for most of traits few times smaller than the family component and this interaction affect was strongly significant for two traits only. The numbers of families contributing to the significance of the interaction effect are indicated in *figure 1*. For total dry weight the ecovalence estimates varied between 2.6% and 6.6%. The corresponding variation for height was 3.4–6.3%. The ecovalence estimates for the 3-way interaction, *population x temperature x watering* treatment, varied between 7.0 and 25.1% for height and between 8.3 and 32.3% for total dry weight.

Table 3. – Means of within-population additive genetic coefficients of variation CV_A (%) of different traits of *Castanea sativa* open-pollinated families by temperature treatment, by watering treatments, by site humidity at location of population's origin, by site humidity and watering treatment combinations, and by country of population's origin. H-height, SDW-stem dry weight, LDW-leaf dry weight, RDW-root dry weight, TDW-total dry weight, RSDW-root/stem dry weight ratio, BRA-branching.

Grouping factors		Mean CV_A (%)						
		H	SDW	LDW	RDW	TDW	RSDW	BRAN
Temperature	25°C	24.39	55.11	43.61	47.56	47.45	17.37	20.98
	32°C	19.50	43.28	34.94	43.93	39.19	14.44	7.62
Watering	Well watered (W)	21.89	49.16	39.59	47.43	43.54	17.41	12.62
	Periodic drought (D)	21.92	49.24	38.96	44.07	43.10	14.39	15.99
Site humidity	Wet	15.70	38.69	30.00	43.06	34.74	13.53	17.23
	Dry	28.02	59.71	48.55	48.44	51.90	18.27	11.37
Site humidity x water treatment	From wet in W	14.04	37.55	29.34	40.79	33.27	11.62	19.78
	From wet in D	17.36	39.83	30.65	45.32	36.20	15.44	14.67
	From dry in W	29.57	60.76	49.84	54.07	53.80	23.21	5.44
	From dry in D	26.48	58.66	47.26	42.81	50.00	13.34	17.30
Country	Greece	26.23	54.34	45.10	36.08	46.39	22.49	8.66
	Italy	24.04	57.29	45.98	60.64	51.50	12.27	10.68
	Spain	15.31	35.96	26.75	40.52	32.06	12.95	23.54

Genetic correlations between the same traits in different treatments were high and significant for most growth and biomass traits. The lowest correlations were observed between treatments T25D (low temperature and drought) and T32D (high temperature and drought, lowest 0.59±0.16) indicating that temperature in the case of drought contributes most to the genotype by environment interaction. Genetic correlations for root to stem ratio and branching were in many cases non-significant owing to low family variance components in some treatments.

Discussion

Experimental precision

The large variation in time of germination and early cessation of growth of several plants suggest that there might be problems with the precision of the estimates of genetic parameters. This was supported by the strongly significant family effect for date of germination and for early heights and by the significant effect on variation of final height and biomass traits. However, the family mean correlation between germination date and total dry weight was negative and weak (r = –0.10 – –0.38) and non-significant in almost all treatments except for the T32W treatment (p = 0.01–0.03). By using germination date as covariate in the analysis of variance this weak correlation became still weaker. The covariate also lowered the significance of the 3-way *family x temperature x watering* interaction effect from p = 0.002 to p = 0.02 for final height while the family effect was unaffected (p = 0.001). The family mean correlation coefficients between height at first registration date (3 weeks after sowing) and total dry weight ranged between 0.32 and 0.65 and they were significant in all treatments. By including the height at 3 weeks of age into the analysis of variances the significance of the family effect and 3-way interaction for final height were both slightly reduced, p = 0.002 instead of p = 0.001 and p = 0.04 instead of p = 0.02, respectively. The growth at three weeks age already indicated the presence of genetic differences between families in growth.

Culling of slower growing seedlings (4%) lowered the significance level of the interaction term somewhat (from p = 0.002 to

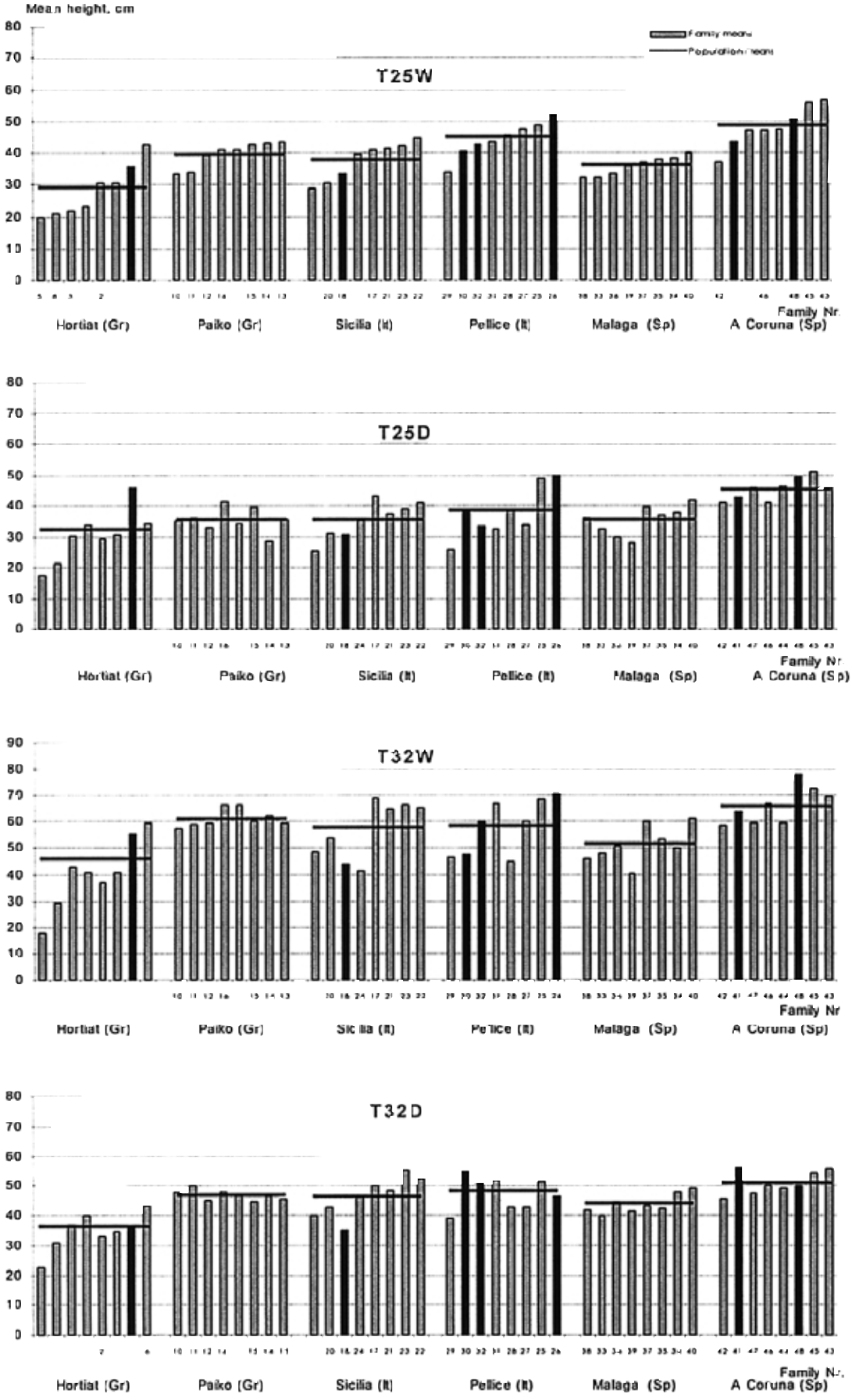

Figure 1. – Mean height of *Castanea sativa* open pollinated families and populations by temperature and watering treatments. Families that contribute significantly ($P < 0.05$) to the interaction variance components are indicated by black bars.

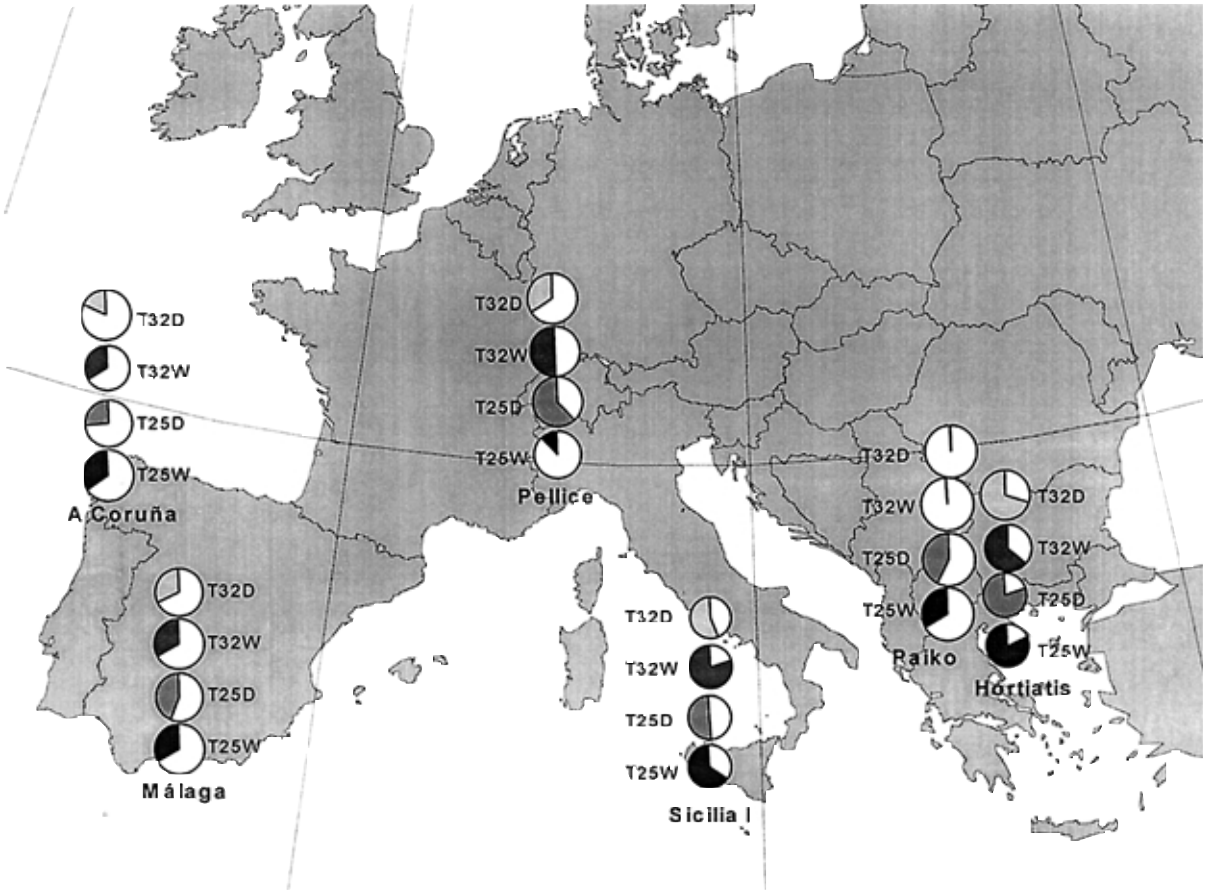

Figure 2. – Additive genetic coefficient of variation (CV_A%) of total dry weight of one-year-old progenies of *Castanea sativa* populations in climatic chambers with different temperature and watering treatments.

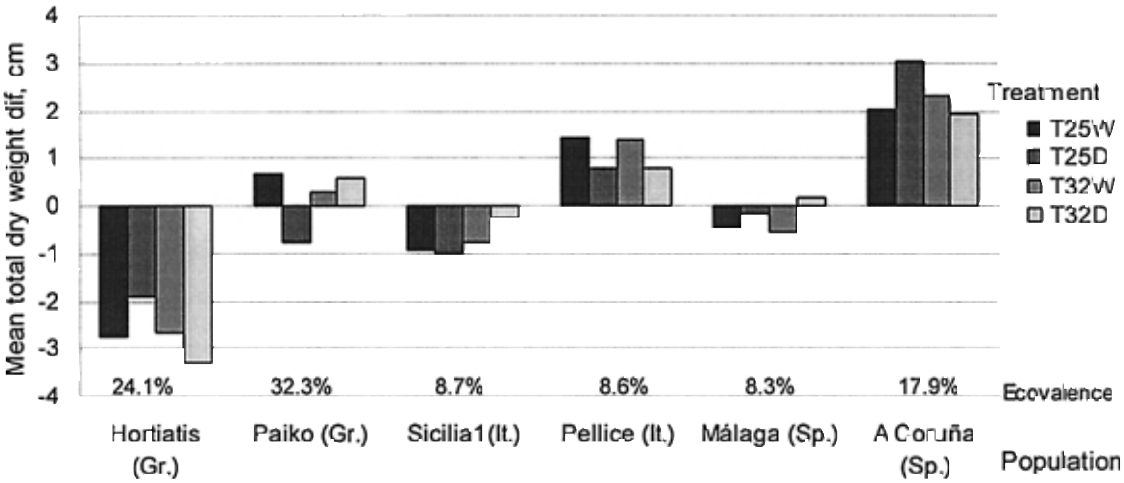

Figure 3. – Variation in total dry weight of *Castaneas sativa* populations in a factorial temperature x watering treatments. Least-squares means values from mixed linear model for individual populations within treatments. Values are scaled to equal population genetic variance within treatments and expressed as deviations from treatment mean. Ecovalence values of each populations are presented.

p = 0.02) while the significance of the family effects remained constant. However, culling resulted in an increase of the means of families and means of populations, and in rank changes of 2–3 families having almost similar mean values within each population.

Based on this discussion we decided to use the interpolated day of germination as covariate and culled seedlings according to methods 1 and 3 described in Material and Methods.

The reason for the weekly measurements of seedling heights was to be able to verify whether or not there was an effect of the drought treatments. If not, the intention was to increase the drought stress to obtain a growth reduction in the treatments with periodic drought. In spite of this we failed to get any pronounced growth reduction in the T25D treatment compared with the growth in the T25W treatment. However, the genetic parameters varied between these two treatments.

Within population variation and its interaction with treatment

As far as we know this is the first study to include within-population estimates of genetic parameters for growth traits in *Castanea sativa*. This excludes comparisons within this species. In agreement with several other studies under controlled conditions the estimates of heritability were high in our experiment (eg. SONESSON and ERIKSSON, 2000). The high estimates of the heritability must partly be attributed to the uniform growth conditions resulting in a low environmental variance. The estimates of CV_A were generally high, which may be attributed to low mean values of the trait under study (mean value = the denominator in the ratio for calculation of CV_A). However, we decline this as an explanation for our high estimates since the mean values were not extremely small in our material. The CV_As were also higher than estimated for other broadleaved tree species (CORNELIUS, 1994 and lit. cit., BALIUCKAS et al., 1999, 2000).

Both the estimates of heritability and CV_A were based on the assumption that the open-pollinated (OP) families were true half-sibs. This can be questioned since there may be some full-sibs included. Trees may be related in naturalised stands and groups of full-sib or half-sib trees may occur. However, to avoid relationship as much as possible the sampled trees grew at a large distance. Still it cannot be excluded that related trees were sampled. Even if we correct for relatedness in our material by multiplying the family variance by 3 instead of 4 our estimates remain high. Repeated mating in small cohorts within each population may lead to an exaggeration of the estimates of heritability and CV_A owing to genetic drift. The wind-pollination of this species speaks against such a hypothesis. Nor do preliminary data from isozyme studies of the same populations support that genetic drift plays any great role (ARAVANOPOULOS, MATTIONI pers. comm.). The isozyme studies over a wider area of species distribution (Turkey - France) indicated a decrease of genetic variation within populations from Eastern Turkey to Europe with the lowest heterozygosity and percentage of polymorphic loci in Italy and France (VILLANI et al., 1994).

As seen from *table 4* there was a variation in the estimates of the heritability and CV_A dependent on the treatment. It is noteworthy that the highest heritability estimates for the growth traits were obtained in the T25D treatment that had poor growth. The two other treatments with similar growth, T32D and T25W, had low estimates of heritability. Even if the growth data of the T25D, T25W, and T32D treatments did not differ much (*Table 4*), the individual populations differed both in means and CV_A for height and total dry weight (cf. *Figs. 1–2*). The differences between these treatments were most pronounced in mean root dry weight, which might have influenced the changes in growth performance both of populations and families within treatments without a change in treatment means. The extremely low CV_As in the T32W and T32D treatments in the mount Paiko population are noteworthy. The low number of families tested (8) in each population does make extremes more likely.

The significant *family x temperature x water* interaction variance components found for height and stem dry weight (*Table 2*) indicated the presence of differences among families in adaptedness to different growth conditions. In spite of this significance the genetic correlation coefficients for the growth traits were 0.70 or higher with three exceptions. These three low coefficients were obtained when T32D was one component in the correlation. The low ecovalence estimates of families indicate that no family was an extreme contributor to the interaction. This means that the stability of the population cannot be improved by culling unstable families.

Among population variation and its interaction with treatment

There was a large variation among populations for the growth traits (*Figure 1* and *4*). The overall best performance of the Coruña population from Spain may partly be attributed to possible presence of hybrids in that naturalised population (FERNÁNDEZ-LÓPEZ, pers. comm.).

Generally isozyme studies of European populations of *Castanea sativa* (cf. Introduction) have revealed limited among-population variation. This contrasts with our findings of significant differences among populations for growth traits and is in agreement with the expectation that adaptive traits show a different structure from neutral markers such as isozymes (FALKENHAGEN, 1985). The most recent isozyme studies of the same populations as used in our study revealed a larger population differentiation than found before (ARAVANOPOULOS, MATTIONI, pers. comm.).

The three-way *population x temperature x water*ing interaction (*Table 2*) indicates the presence of some specific adaptations. However, the changes in growth were of irregular pattern and were related to different combinations of temperature and watering. In Turkey two physiological types of populations were identified, drought adapted and wet adapted (VILLANI et al., 1999b; LAUTERI et al., 1999). Lower juvenile above ground growth was found for drought-adapted Mediterranean geno-

Table 4. – Mean values, individual heritabilities, standard errors and additive genetic coefficients of variation of different traits of *Castanea sativa* open-pollinated families for individual treatments: T25W-normal temperature and well watered, T25D-normal temperature and periodic drought, T32W-high temperature and well watered, T32D-high temperature and periodic drought.

Trait	Treatment	Mean	Family additive	
			h_i^2 ±se	CV_A (%)
Height (H), cm	T25W	39.67	0.51 ±0.14	21.7
	T25D	37.58	0.89 ±0.18	28.3
	T32W	57.76	0.76 ±0.17	24.3
	T32D	46.32	0.48 ±0.13	16.2
Stem dry weight (SDW), g	T25W	1.84	0.42 ±0.13	43.9
	T25D	1.79	0.81 ±0.18	56.6
	T32W	2.91	0.66 ±0.16	51.8
	T32D	1.87	0.43 ±0.13	35.6
Leaf dry weight (LDW), g	T25W	3.52	0.50 ±0.14	39.1
	T25D	3.40	0.69 ±0.16	42.9
	T32W	4.19	0.56 ±0.15	40.1
	T32D	3.23	0.45 ±0.13	30.8
Root dry weight (RDW), g	T25W	1.29	0.48 ±0.14	48.3
	T25D	1.44	0.57 ±0.15	46.6
	T32W	1.38	0.49 ±0.14	51.4
	T32D	1.18	0.50 ±0.14	41.7
Total dry weight (TDW), g	T25W	6.66	0.49 ±0.14	41.3
	T25D	6.62	0.75 ±0.17	46.8
	T32W	8.47	0.62 ±0.16	45.7
	T32D	6.28	0.47 ±0.13	33.6
Root/stem ratio (RSDW)	T25W	0.810	0.19 ±0.09	21.7
	T25D	0.927	0.08 ±0.07	17.4
	T32W	0.537	0.14 ±0.09	19.8
	T32D	0.671	0.10 ±0.08	12.3
Root/leaf ratio (LSDW)	T25W	0.406	0.18 ±0.09	27.7
	T25D	0.466	0 ±0	0
	T32W	0.348	0.08 ±0.08	23.5
	T32D	0.365	0.34 ±0.12	21.6
Branching (BRAN), points	T25W	1.49	0.27 ±0.11	26.0
	T25D	1.70	0.32 ±0.11	28.9
	T32W	1.42	0.11 ±0.08	15.3
	T32D	1.38	0.05 ±0.05	10.6

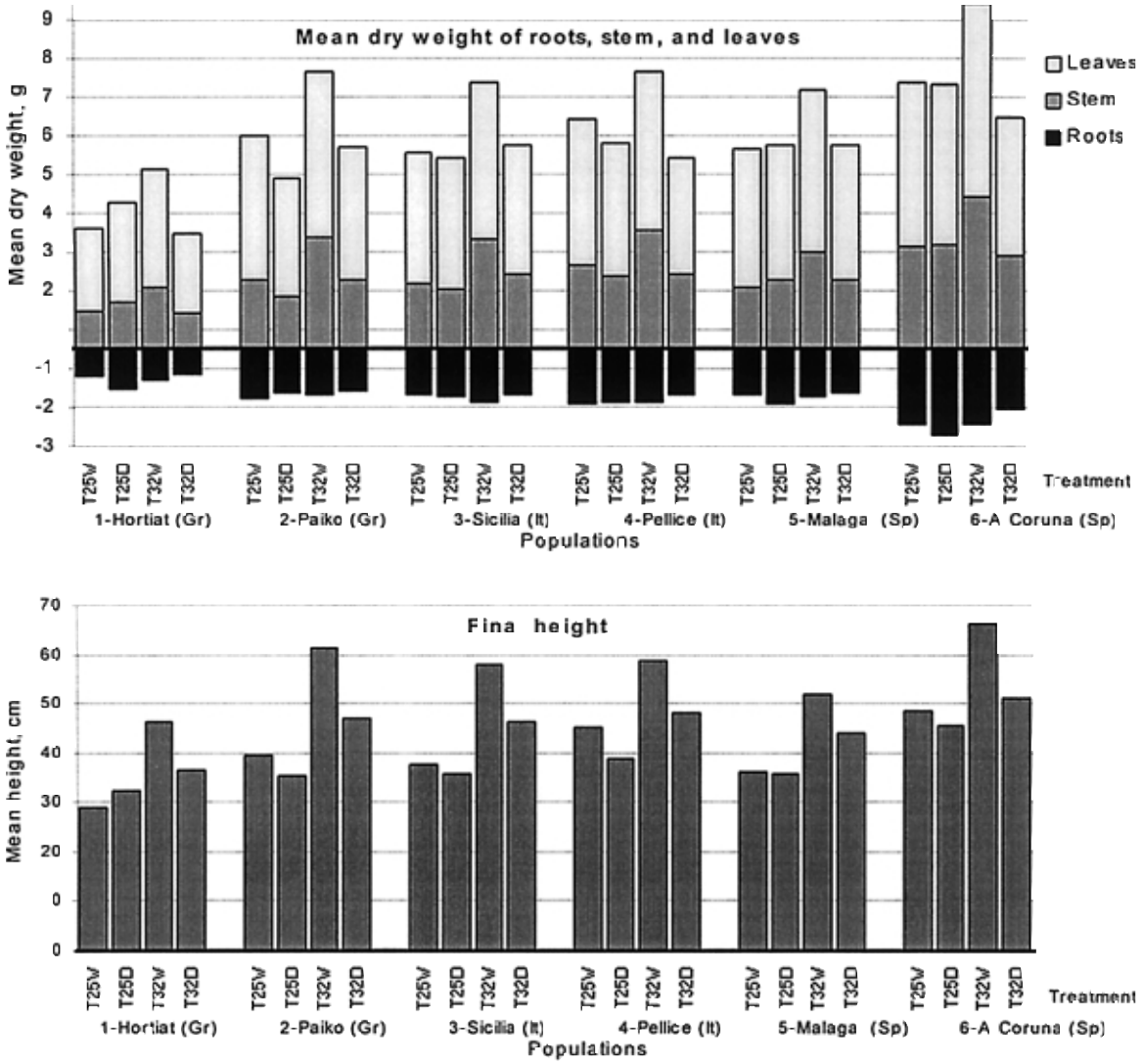

Figure 4. – Distribution of biomass and height of progenies from *Castanea sativa* populations by temperature x watering treatments.

types than in eastern wet-adapted ones with inversion of growth patterns in adult stages (VILLANI et al., 1992; LAUTERI et al., 1997). However, in our experiment under the drought treatments the progenies from presumably drought-adapted Mediterranean populations Hortiatis, Paiko, and Sicilia did not develop bigger root systems. Moreover, their root/shoot ratios were not increased more than in the populations originating from wet regions. The presence of specific adaptations should be considered with some reservations because of precision problems in estimation of *g x e* interaction component in joint ANOVA due to heterogeneous error variance in different treatments.

Consequences for gene conservation

The prime objective of gene conservation of *Castanea sativa* is to safeguard the potential for adaptation and to encompass the existing adaptedness in the gene resource populations (ERIKSSON, 2001). For gene conservation our data are one part out of many needed for a genetically solid gene conservation programme. The objective of safeguarding the potential for adaptation is particularly relevant during rapidly changing climatic conditions. To match this objective, the adaptability should be maximised to allow for natural selection, or for artificial selection by breeders and gene conservationists. The most intensive selection may take place during the juvenile phase. Large additive variance for height growth and biomass traits found within the six natural/naturalised populations of *Castanea sativa* suggests that there are good prospects for these populations to respond to natural selection and thus have a high potential for adaptation to future climates. With the variation among populations observed in the present study as well as earlier observations (VILLANI and PIGLIUCCI, 1991; LAUTERI et al., 1999) the best solution for a dynamic gene conservation is to apply the *Multiple Population Breeding System,* MPBS, concept developed for joint breeding and gene conservation by NAMKOONG (1984). Our suggestion for gene conservation of *Castanea sativa* is in agreement with the suggestions by FERNÁNDEZ-LÓPEZ and ALIA (1999) and ERIKSSON (2001).

A large phenotypic plasticity in adaptive traits may be useful under changing conditions in a short-time perspective but may be detrimental in a long-term perspective since it means that natural selection cannot operate at full strength (cf ERIKSSON,

2000). The significant treatment effects indicate high phenotypic plasticity in our study. This means that individual trees in the populations have the ability in the short term to respond to climatic changes within the range of treatments in our study.

Conclusions

This is the first study of variation in juvenile growth traits in *Castanea sativa* under controlled growth conditions. There were strong treatment effects in this 2 x 2 factorial *temperature x water* availability experiment. The amount of additive variance in all populations was high but it varied among treatments. This suggests that these populations have high potential for adaptation during the juvenile stage if environmental conditions change. There was significant variation among the six populations for most traits, which suggests that gene conservation should encompass all six populations according to the multiple population breeding system concept. The significant *family x treatment* and *population x treatment* interactions mean that populations and families responded differently to the treatments. It was striking that the contribution of individual families to the interaction was low, not exceeding 7%, and that even a family with such a small ecovalence as 2.6% contributed significantly to the interaction.

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References

BALIUCKAS, V., EKBERG, I., ERIKSSON, G. and NORELL, L.: Genetic variation among and within populations of four Swedish hardwoods species assessed in a nursery trial. Silvae Genetica **48**, 1, 17–25 (1999). — BALIUCKAS, V., LAGERSTRÖM, T. and ERIKSSON, G.: Within- and among population variation in juvenile growth rhythm and growth in *Fraxinus excelsior* and *Prunus avium.* Forest Genetics **7** (3), 193–202 (2000). — BECKER, W. A.: Manual of quantitative genetics. Ed. 4. Academic Enterprices, Pullman, WA., 188 pp. (1984). — CARSON, H. L.: Increasing genetic variance after a population bottleneck. Trends in Ecology and Evolution 5, 228–231 (1990). — COLINAS, C., USCUPLIC, M. and SALESSES, G.: Studies on chestnut blight (*Cryphonectria parasitica* (Murr.) Barr) in north-east Spain. Proceedings of the 2nd International Symposium on Chestnut, Bordeaux, France, 19–23 October 1998. Acta-Horticulturae **494**, 495–500 (1999). — CORNELIUS, J.: Heritabilities and additive genetic coefficients of variation in forest trees. Can. J. For. Res. **24**, 372–379 (1994). — DANELL, Ö.: Arbetsgång vid bearbetning av contortaförsök. Inst. for For. Improve. Arbetrapport 219. Uppsala. (in Swedish) (1988). — ELLSTRAND, N. C.: Gene flow by pollen: implications for plant conservation genetics. Oikos **63**, 77–86 (1992). — ERICSSON, T.: Lodgepole pine (*Pinus contorta* var latifolia) breeding in Sweden – results and prospects based on early evaluations. Ph. D. thesis, Dep. of For. Gen. and Plant Physiol., Swedish Univ. of Agric. Sci., Umeå, Sweden. 32 pp. (1984). — ERIKSSON, G.: To survive or not survive under global warming? In.: International collaboration on forest genetic resources: the role of Europe. Proc. EUFORGEN 2nd Steer. Comm. Meet. 26–29 Nov., 1998 Vienna, Austria, (Eds. J. TUROK and TH. GEBUREK), 36–43 (2000). — ERIKSSON, G.: Conservation of noble hardwoods in Europe. Can. J. For. Res. **31**, 577–587 (2001). — ERIKSSON, G., NAMKOONG, G. and ROBERDS, J.: Dynamic gene conservation for uncertain futures For. Ecol. Managem. **62**, 15–37 (1993). — FERNÁNDEZ-LÓPEZ, J. and ALIA, R.: *Castanea sativa*. In: Noble Harwoods network. Report of the 3rd Meeting, 13–16 June, Sagadi, Estonia. (Eds. J. TUROK, J. JENSEN, CH. PALMBERG-LERCHE, M. RUSANEN, K. RUSSEL, S. DE VRIES, and E. LIPMAN), p. 21–27 (1999). — FALKENHAGEN, E. R.: Isozyme studies in provenance research of forest trees. Theor. Appl. Genet. **69**: 335–347 (1985). — GILMOUR, A. R., CULLIS, B. R., WELHAM, S. J. and THOMPSON, R.: ASREML software. New South Wales Agriculture. Australia. (2000a). — GILMOUR, A. R., CULLIS, B. R., WELHAM, S. J. and THOMPSON, R.: ASREML Program user manual. New South Wales Agriculture. Australia, 218 p. p. (2000b). — GILPIN, M.: The genetic effective size of a metapopulation. Biological Journal of the Linnean Society **42**, 165–175 (1991). — HUNTLEY, B. and BIRKES, H. J. B.: An atlas of past and present pollen maps for Europe: 0-13000 years ago. Cambridge University Press, Cambridge, UK (1983). — INGESTAD, T. and LUND, A. B.: Theory and techniques for steady state mineral nutrition and growth of plants. Scand. J. For. Res. 1, **4**, 439–453 (1986). — KATTENBERG, F., GIORGI, F., GRASSL, H., MEEHL, G. A., MITCHELL, J. F. B., STOUFFER, R. J., TOKIOKA, T., WEAVER, A. J. and WIGLEY, T. M. L.: Climate models – projections of future climate. In Climate change 1995, The Science of Climate Change (Eds. J. T. HOUGHTON, L. G. MEIRA FILHO, B. A. CALLANDER, N. HARRIS, A. KATTENBERG and K. MASKELL), 289–357 (1996). — KARHU, A., HURME, P., KARJALAINEN, M., KARVONEN, P., KÄRKKÄINEN, K., NEALE, D. and SAVOLAINENE, O.: Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? Theor. Appl. Genet. **93**, 215–221 (1996). — LAUTERI, M., MONTEVERDI, M. C., SANSOTTA, A., KÜÇÜK, M., CHERUBINI, M., SPACCINO, L. and VILLANI, F.: Adaptation to drought in european chestnut. evidences from a hybrid zone and from controlled crosses between drought and wet adapted populations. Proc. 2-nd. Int. Symp. on Chesnut. (Ed. G. SALESSES) Acta Horticulturae **494**, 345–353 (1999). — LAUTERI, M., SCARTAZZA, A., GUIDO, M. C. and BRUGNOLI, E.: Genetic variation in photosynthetic capacity, carbon isotope discrimination and mesophyll conductance in provenances of *Castanea sativa* adapted to different environments. Functional Ecology **11**, 675–683 (1997a). — LAUTERI, M., MONTEVERDI, M. C., SCARTAZZA, A., AUGUSTI, A., BRUGNOLI, E., SPACCINO, L. and CHERUBINI, M.: Stable isotope and forest ecophysiology. Two case studies concerning: a) adaptation of *Castanea sativa* MILL. to contrasting environments; b)seasonal variation of water use efficiency within and among plant communities of a mediterranean coastland ecosystem. S.I.S.E.F proceedings **1**, 303–307 (1997 b). — NAMKOONG, G.: A control concept of gene conservation. Silvae Genetica **33**, 160–163 (1984). — RITLAND, K.: Marker-based method for inference about quantitative inheritance in natural populations. Evolution **50**, 1062–1073 (1996). — SAS Institute Inc. SAS/STAT® software. Release 6.12. SAS Institute Inc., Cary, NC (1997). — SHUKLA, G. K. Some statistical aspects of partitioning genotype-environment components of variability. Heredity **29**, 237–245 (1972). — SLATKIN, M.: Gene flow and the geographic structure of natural populations. Science **236**, 787–792 (1987). — SONESSON, J. and ERIKSSON, G.: Genotypic stability and genetic parameters for growth and biomass traits in a water x temperature factorial experiment with *Pinus sylvestris* L. seedlings. For. Sci., **46**, 479–495 (2000). — VILLANI, F., LAUTERI, M., SANSOTTA, M., CHERUBINI, M., MONTEVERDI, M. C., MATTIONI, C., CASASOLI, M. and KÜÇÜK, M.: Genetic structure and quantitative traits variation in F1 full-sibs progenies of *Castanea sativa* MILL. Proc. 2-nd. Int. Symp. on Chestnut. Ed. G. SALESSES. Acta Horticulturae **494**, 395–405 (1999a). — VILLANI, F., SANSOTTA, A., CHERUBINI, M., CESARONI, D., and SBORDONI, V.: Genetic structure of natural populations of *Castanea sativa* in Turkey: evidence of a hybrid zone. Journal of Evolutionary Biology **12**, 233–244 (1999b). — VILLANI, F. and PIGLIUCCI M.: Origin and evolution of European chestnut: a population biology perspective. In: Genetic variation in European populations of forest trees. (Eds. G. MÜLLER-STARCK and M. ZIECHE) Sauerländers Verlag, Frankfurt am Main, 173–179 (1991). — VILLANI, F., PIGLIUCCI M. and CHERUBINI, M.: Evolution of *Castanea sativa* MILL. in Turkey and Europe. Genetic Research, **63**, 109–116 (1994). — VILLANI, F., PIGLIUCCI, M., LAUTERI, M., CHERUBINI, M. and SUN, O.: Congruence between genetic, morphometric and physiological data on differentiation of Turkish chestnut (*Castanea sativa* MILL.). Genome, **35** (2), 251–256 (1992). — WRICKE, G.: Übereine methode zurerfassung der ökologischen streubreite in feldversuchen. Z. Pflantzenzucht. **47**: 92–96 (1962, in German). — XENOPOULOS, S. G., PAPACHATZIS, A. and SALESSES, G.: Problems of chestnut-growing in Greece screening for resistance of several chestnut provenances to *Cryphonectria (Endothia) parasitica* (Murr.) Aderson. Proceedings of the 2nd Int. Symp. on Chestnut, Bordeaux, France, 19–23 October 1998. Acta-Horticulturae, **494**, 521–527 (1999). — YOUNG, A., BOYLE, T. and BROWN, T.: The population genetic consequences of habitat fragmentation in plants. Trends in Ecology and Evolution **11**, 413–419 (1996).