# First surveys on genetic variability and structure of field maple (*Acer campestre* L.) in natural and managed populations in the landscape of central and southern Italy [§]

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Accettato il 23 novembre 2010

**Abstract –** Four Tuscan populations (central Italy) and three Italian populations from southern Italy (Campania) were sampled to compare their genetic variability and genetic structure. In each geographical area one of the sampled populations is originated naturally in forest and used as a local reference. The remaining populations were originated artificially. Indeed, field maple was traditionally used in Italy to supply fresh fodder to animals in dry summer period as tree twigs or to train up grape trees in the fields edges. This tradition initiated at the time of Etruscans and continued throughout the Roman partitioning of agriculture landscape. Biochemical markers were used to explore variability in the examined populations (5 enzyme systems by 11 *loci*). Results showed that the main amount of variation is due to the individual component as for most of the scattered hardwoods in Europe and that differentiation among populations for these neutral traits is relatively low. On the other hand, the natural populations in both the geographical areas showed a very high level of panmittic equilibrium, whilst the artificial populations were really distant from this condition showing a high probability of "founder effect". This could be determined by the former system of self-supplying reproductive material carried out by farmers, based on the wild offspring collection growing around few mother trees. Discussion is focused also on how handling the opportunity given by many hundreds kilometers of lines in the agriculture landscape as a way of managing diversity for this species.

**Key words:** Acer campestre*, isozymes, genetic variation, conservation, Tuscany, Campania.*

**Riassunto** – Prime indagini sulla variabilità e la struttura genetica dell'acero campestre (*Acer campestre* L.) in ambienti influenzati dalla presenza antropica in Italia centrale e meridionale. Questo lavoro compara la variabilità e la struttura genetica di alcune popolazioni dell'Italia centrale (4 in Toscana, in provincia di Arezzo) e di altre campionate in Campania (2 in provincia di Salerno ed 1 in provincia di Caserta). Di queste, alcune sono di origine artificiale, tradizionalmente rappresentate da filari, maritate alle viti, sui bordi delle antiche centuriazioni romane, o semplicemente costituite da piante usate ai bordi di campi o antichi pascoli per fornire frasca verde al bestiame nei periodi estivi. Altre sono invece di origine naturale, cresciute in foresta a bassa influenza antropica, come quelle di Dagnano (Arezzo) e di Cerreta (Salerno), usate per questo studio come riferimenti locali. L'analisi genetica è stata effettuata con marcatori di tipo biochimico (5 sistemi isoenzimatici che hanno consentito di individuare 11 *loci*). L'indagine ha come obiettivo quello di verificare l'eventuale impatto della gestione del paesaggio agricolo e della sua storia sulla strutturazione della diversità genetica e quale potrebbe esserne la migliore gestione. La specie ha rivelato, come per altre a distribuzione sparsa delle foreste europee ed italiane, livelli di differenziazione relativamente bassi ed una elevata componente individuale della variabilità. Le popolazioni artificiali hanno inoltre mostrato le probabili conseguenze del sistema di approvvigionamento adottato dai contadini per rifornirsi di materiali di propagazione. Esso consisteva probabilmente nel prelievo di "selvaggioni" ai piedi di poche o singole piante madri, in bosco o in filari più antichi, per il quale i dati raccolti lasciano intuire un probabile "effetto del fondatore". Per contro, le popolazioni di riferimento di origine naturale mostrano elevata eterozigosi, con equilibri prossimi alla panmissia. L'acero campestre non è, sicuramente, una specie a rischio, ma costituisce una caratteristica storica importante del paesaggio toscano e del centro-Italia in genere che andrebbe conservata. I filari presenti su tutto il territorio sono, infatti, una riserva genetica rilevante. Di conseguenza, sarebbe utile e auspicabile che le filiere vivaistiche venissero impostate tenendo conto anche di questo esempio per favorire una gestione sostenibile del paesaggio (in questo caso interessato da centinaia di chilometri di filari) e per tutelare e conservare la diversità.

**Parole chiave:** Acer campestre, *isoenzimi, variabilità genetica, conservazione, Toscana, Campania.*

F.D.C.: 176. 1 Acer campestre: 165: (450. 5/.7)

## Introduction

Field maple (*Acer campestre* L.) is a diployd species (2n = 26) belonging to *Aceraceae* (Gellini and Grossoni 1997). In Italy there are at least 6 spontaneous species of the genus *Acer*, distinguishable mainly for the different characters of leaves and fruits: *Acer platanoides* L. (Norway maple), *Acer lobelii* Ten.

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[§] Research framed within the programme "Forest nurseries productions in the regional structures - Identification of basic materials", carried out in agreement between the Region of Campania and CRA (Council for Research and Experimentation on Agriculture), coordinated by F. Ducci

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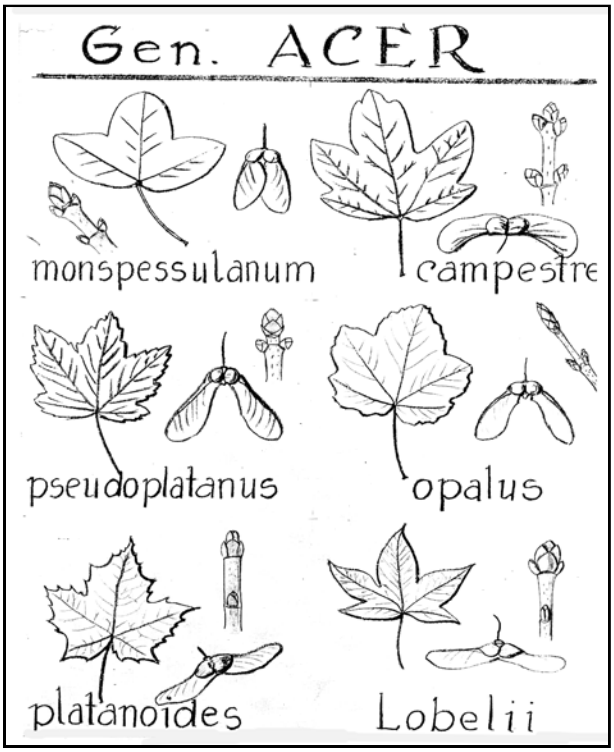
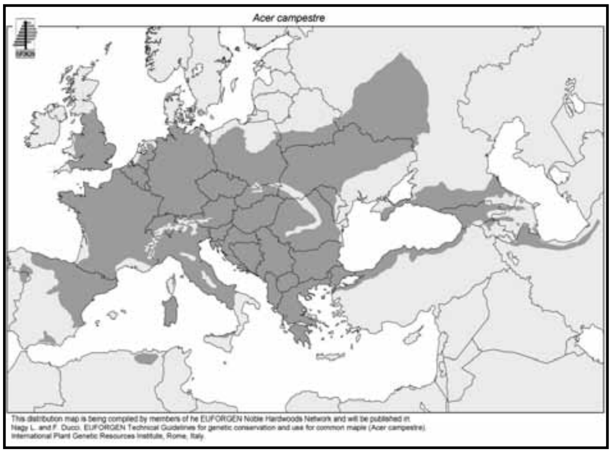


**Fig. 1** - Species of the genus Acer spontaneous in Italy (Allegri 1988).
*Le specie spontanee di acero in Italia (Allegri 1988).*



This distribution map is being compiled by members of he EUFORGEN Noble Hardwoods Network and will be published in: Nagy L. and F. Ducci. EUFORGEN Technical Guidelines for genetic conservation and use for common maple (Acer campestre). International Plant Genetic Resources Institute, Rome, Italy.

**Fig. 2** - Natural distribution range of *Acer campestre* (Nagy and Ducci 2003).
*Areale naturale di* Acer campestre *(Nagy e Ducci 2003).*

(Lobel maple), *Acer campestre* L., *Acer pseudoplatanus* L. (sycamore maple), *Acer monspessulanum* L. (Montpellier maple) and *Acer opalus* Mill. (Italian maple; Figure 1).

*A. campestre* has small leaves, generally with 5 lobes, and produces flowers in corymbs. Morphologically, field maple is hermaphroditic, but functionally unisexual due to reduction to one sex. Thus, it can predominantly be male or female. Pollination is entomophilous, with possibility of self-fertilization. Its fruits (double-winged samaras with opposing wings) ripen in September and are dispersed by the wind.

The natural distribution of the field maple covers most of Europe (Nagy and Ducci 2003), till Vistula and Don rivers (latitudinal range: 55°N to 38°N), but it is absent in Scotland and Scandinavia and rare in southern Spain. It is also present in northern Africa and Turkey, up to the Caucasus and Iran (Figure 2).

*A. campestre* is very common in all Italian regions and with *Carpinus betulus* L., *Ostrya carpinifolia* Scop. and *Fraxinus ornus* L., it forms the lower canopy of xerothermic oak forests (Bernetti 1995; Gellini and Grossoni *op. cit.*). Field maple can be found in central Italy traditionally used as living props for grapevines (hence the common name field maple) on hedges and along the margins of abandoned fields where it can become an invasive species. Since the species can tolerate pruning and defoliation, in the past it was used as green fodder in summer dry period for animals.

It is a post-pioneer species with wide ecological range; it is not possible to find pure populations of field maple, but only scattered plants or small groups.

Because of the utilization in agriculture since 10,000 years, the field maple has been strongly manipulated. The long cultivation history especially in central Italy has contributed to shape the agricultural landscape. Indeed, as early as the 9th century BC (Photo 1), Etruscans and Greeks slowly began to replace burnbeating with fallow and use shrubs and tree cultivation in a systematic way (Sereni 1982).

**Photo 1** - Fields delimited by field maple lines standing up grapes trees, at the Latin use, encircled the walls of ancient towns (Arezzo, Tuscany).
*I campi delimitati alla maniera latina da filari di acero campestre maritati alla vite arrivavano fino alle mura delle città (Arezzo, Toscana).*

**Photo 2** - It was of common use in central Italy standing up wine grapes trees with hardwoods, mainly to field maple (*Acer campestre*).
*Era di uso comune maritare la vite principalmente all'acero campestre in Italia centrale.*

The promiscuous cultivation (cereals with fruit trees) adopted by the Etruscans contributed more than other factors to differentiate landscape and, indirectly, to spread field maple. In this system, grapevines were indeed trained up to tree species that could withstand defoliation and pruning, like *A. campestre* (Photo 2). The cultivation of vineyards with "grapevines trained up to field maple trees" was so widespread that, in the subsequent Roman age, field maple was defined as *arbustum gallicum* and was used to divide in *centuriae* the conquered territories.

Field maple was therefore an important element in the agricultural landscape until the first half of the twentieth century when, with the spread of mechanization, promiscuous crops were replaced by specialized crops. This change contributed not only to reshape the rural landascape, but it also had an influence on the genetic resources of field maple.

Unlike other forest species - economically more important - studies on genetic variability of field maple are limited, even though it has an important role both from ecological and naturalistic point of view and as significant component of agricultural biodiversity that should be known, protected and preserved. Furthermore, due to its characteristics of spreading, genetic resources of field maple are put at risk by human activities, by the adoption of silvicultural techniques privileging major species in forests and by genetic introgression.

Unfortunately, in Italy there are only few studies concerning the genetic diversity of genus *Acer* (Belletti and Monteleone 2000; AA. VV. 2003; Belletti *et al.* 2004 and 2007) and the genetic variability or the amount of resources currently available is still unknown. No specific studies were recorded on *A. campestre.*

This research aims to make an initial survey of field maple resources still present in central Italy by the analysis of the genetic variation in several selected populations. Genetic variation enables species to survive and adapt to changing environment. For this reason measurement and characterization of this variation - particularly in relation to human activities - is the first step to develop a management strategy designed to maintain stable, productive and sustainable forest populations (Hamrick *et al.* 1992; Rusanen *et al.* 2000).

The analysis of genetic variability was made with biochemical markers. Isozymes are less informative than molecular markers, because of the smaller number of identified *loci* and lower degree of polymorphism observed (Rothe 1990), but they are codominant, require shorter time and smaller lab costs compared to molecular markers. Consequently, it is possible to obtain useful information in the initial study of population genetic structure (Geburek 1997; Müller-Starck 1998; Finkeldey 2001). Concerning genus *Acer*, well-performed surveys realized with isozymes can be found in literature in the last few years (Rusanen *et al. op. cit.*; Konnert *et al.* 2001; Eriksson *et al.* 2003; Rusanen *et al.* 2003; Iddrisu and Ritland 2004; Belletti *et al. op. cit.*).

Although some authors have disputed this assertion (Bergmann and Gregorius 1993; Howe *et al.* 2003), isozymes are considered neutral markers (Kremer *et al.* 2002). Consequently, isozyme expression is not conditioned by factors determining natural selection, as it happens with non-neutral markers such as phenotypic traits. However, although not directly related to adaptive characters, isozymes constitute a useful tool for management and conservation of genetic resources (Barreneche *et al.* 1998; Streiff *et al.* 1998; Gömöry *et al.* 2001; Finkelday and Màtyàs 2003).

## Materials and methods

### *Plant material and sampling*

Since the field maple tree is a sporadic and marginal the traditional concept of population cannot be applied. Indeed, the maple individuals present a different scattering in respect to other social species that cover wide, continuous areas where an adequate sample of trees is possible. Therefore for this study,

**Tab.1** - Details of the main stand characteristics of field maple (*Acer campestre*) populations sampled for this study.
*Principali caratteristiche delle popolazioni di acero (*Acer campestre*) campionate per questo studio.*

| Population | Location | | Elevation (m above sea level) | Mean annual rainfull (mm) | Pavari phytoclimatic zone | Stand |
|---|---|---|---|---|---|---|
| | Mean Lat. (°N) | Mean Long. (°E) | | | | |
| Dagnano (Ar) | 43° 39’ | 12° 03’ | 480 | 1000-1200 | *Lauretum,* cold sub-zone; *Castanetum* | Scattered trees on margins of the forest |
| Pieve (Ar) | 43° 40’ | 12° 02’ | 480 | 1200-1300 | *Lauretum* cold sub-zone; *Castanetum* | Scattered trees on margins of the fields |
| Ca’ de Cìo (Ar) | 43° 29’ | 11° 54’ | 260 | 800-900 | *Lauretum* medium sub-zone | Rows on the sidelines of the fields |
| Chiana (Ar) | 43° 27’ | 11° 44’ | 300 | 800-900 | *Lauretum* medium and cold sub-zone; *Castanetum* | Scattered trees on margins of the fields |
| Vesolo (Sa) | 40° 18’ | 15° 35’ | 650 | 1200-1400 | *Castanetum* | Scatterd trees around the edges of the forest |
| Cerreta (Sa) | 40° 15’ | 15° 41’ | 550 | 1200-1400 | *Lauretum* medium and cold sub-zone | Scatterd trees around the edges of the forest |
| Baia (Ce) | 41° 19’ | 14° 14’ | 100 | 900-1000 | *Lauretum* hot sub-zone | Scattered trees on the margins of the fields |

only populations consisting of at least 10 individuals were considered. In populations sampled on the margins of forests, the chosen plants were at a distance of least 50 m one from the other, while in old vineyards all field maple trees were sampled.

Within the Arezzo (Ar) province (Tuscany), populations were sampled in the following localities: Dagnano, Pieve, Ca' de Cìo  and Chiana. Scattered plants were sampled on the margins of fields, or trees inside old vineyards where the landscape is still influenced by the former Roman division. The main characteristics of the examined populations are listed in Table 1.

Three populations sited in Campania region were also sampled and used as a comparision sample: Baia (Caserta - Ce), Vesolo and Cerreta (Salerno - Sa). Among both groups a population is growing in forest (Table 1). This characteristic permitted to compare the genetic diversity between plants artificially originated (*e.g.* trees sampled in vineyards) with "natural” populations (*e.g.* trees sampled in forest). The geographic distribution of the seven analysed populations is given in Figure 3.

Winter buds were sampled from all trees and after collection they were stored at -80°C until protein extraction.

### *Electrophoretic procedures*

Since it is not possible to provide information about the genetic variability of *A. campestre* in Italy, the research carried out by Leinemann and Bendixen (1999) was used as reference since it represents the first study on genetic control of isozymatic *loci* in field maple.

Enzymes were separated by means of horizontal starch gel electrophoresis in 11.5% hydrolysed potato starch gel (Sigma S4501). Protein extraction and gel and electrode buffers composition were the ones proposed by Leinemann and Bendixen (*op. cit.*) and Bendixen (2001) modified. Stain procedures were carried out according to Cheliak and Pitel (1985) and Vallejos (1983). The analyzed enzymatic systems, identified *loci* and allele variants are shown in Table 2. The most anodal zone of each *locus* was designated with number 1. Within a single zone of activity alleles were labelled with lower-case letters (*a* for the fastest migrates band).

### *Data analysis*

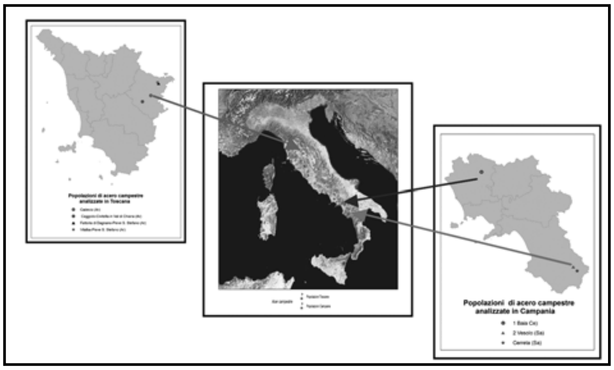


**Fig. 3** - Geographic distribution of the seven populations of field maple analysed in this study.
*Localizzazione delle sette popolazioni di acero campestre analizzate.*

The main indices of genetic variation - such as mean and effective number of alleleles per *locus* (A and $A_e$ respectively), allele frequencies, percentage of polymorphic *loci* ($P_{5\%}$)[3] , observed heterozygosity ($H_o$) and Nei's (1978) gene diversity ($H_e$) - were estimated for Tuscan populations and for the reference system using Biosys-1 software package (Swofford and Selander 1989). The Evens-Watterson neutrality test (Watterson 1978) was performed on each *locus* to test the neutrality of allele distribution[4], using the algorithm of Manly (1985; with 1000 permutations). This index was assessed with the POPGENE version 1.31 software package (Yeh *et al.* 1999). Chi-square ($\chi^2$) and likelihood ratio ($G^2$) tests were performed on allele frequencies to check significant differences between populations with respect to Hardy-Weinberg equilibrium (Goudet 2001). The departure from the Hardy-Weinberg proportions and linkage disequilibrium were investigated using probability exact tests (Guo and Thompson 1992), provided in GENEPOP version 4.0.7 software package (Rousset 2008). A Fisher test was performed to compute unbiased estimates of the exact probabilities (p-values). Furthermore, concerning genetic differentiations, significance of differences in allele frequencies between samples were determinated by using the Fisher's exact test with the Markov chain method (Markov chain parameters: 10,000 dememorization; 100 batches, 5,000 iterations per batch).

**Tab. 2** - Enzyme systems used in the study and allelic variants identified.
*Sistemi enzimatici analizzati nello studio e varianti alleliche identificate.*

| Enzyme | Acronym | EC code | *Loci* N. | Allelic variants |
|---|---|---|---|---|
| Alcohol dehydrogenase | ADH | 1.1.1.1 | 2 | ADH-2a<br>ADH-2b<br>ADH-2c |
| Glutamate oxaloacetate transaminase | GOT | 6.2.1.1 | 3 | GOT-2a<br>GOT-2b<br>GOT-2c<br>GOT-3a<br>GOT-3b<br>GOT-3c |
| Isocitric dehydrogenase | IDH | 1.1.1.42 | 1 | IDH-1a<br>IDH-1b |
| Phosphoglucomutase | PGM | 2.7.5.1 | 3 | PGM-2a<br>PGM-2b<br>PGM-3a<br>PGM-3b<br>PGM-3c |
| Phosphoglucose isomerase | PGI | 5.3.1.9 | 2 | PGI-2a<br>PGI-2b<br>PGI-2c<br>PGI-2d<br>PGI-2e |
| Leucine aminopeptidase | LAP | 3.4.11.1 | 2 | - |

The FSTAT version 2.9.3.2 software package (Goudet *op. cit.*) was used to measure the departures of genotypic frequencies from Hardy-Weinberg expectation, or inbreeding coefficient [$F=1-(H_o/H_e)$] (Wright 1978), and to estimate the genetic differentiation among populations (Nei 1987; Hartl and Clark 1989). The latter was estimated by breaking down the total genetic variability ($H_T$) in its intra-population ($H_S$) and inter-population ($D_{ST}$) component. The genetic differentiation level was calculated according to the following ratio: $G_{ST} = D_{ST}/ H_T$. Besides, the F-statistics were estimated (Weir and Cockerham 1984) describing the genetic structure of populations: $F_{IT}$ (genetic variability in total population), $F_{ST}$ (component of genetic variability between populations) and $F_{IS}$ (component of genetic variability within each population). Both groups of indices estimate genetic differentiation among populations. They are fixation indices evaluating any excess or defect of homozygotes compared to reference values. In order to reduce the effect of missing data and/or any sampling defects (Weir and Cockerham *op. cit.*; Cavalli-Sforza *et al.* 1994), the FSTAT program was used to carry out the bootstrapping method (Raymond and Rousset 1995).

The matrix of unbiased genetic distances (Nei 1978) was used to obtain UPGMA dendrogram (Unweighted Pair-Group Method with Arithmetic averaging; Sneath and Sokal 1973) which divide the populations in clusters, verifying the possible geographic structure of genetic information (Farris 1972).

The degree of genetic isolation (gene flow) was estimated by $N_m$ (number of migrants per generation), through two methods:

1) by the relationship between $F_{ST}$ and $N_m$. In this case from Wright (1951): $N_m = (1-F_{ST})/4F_{ST}$, where $F_{ST}$ was the proportion of the total genetic diversity among populations;
2) by the method of private alleles (unique alleles found in only one population). In this case $N_m$ was assessed by GENEPOP version 4.0.7 (Rousset *op. cit.*), using the frequency and distribution of rare

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[3] By convention, a *locus* was considered polymorphic when the frequency of the most common allele was less than 95%. The value of $P_{5\%}$ was computed considering both monomorphic and polymorphic *loci*.

[4] A *locus* was considered neutral when its mean E-W value fell within the lower and the upper limits of 95% confidence interval of expected F value.

allele among populations, as developed by Slatkin (1985) and Barton and Slatkin (1986).

The main components analysis of the dispersion matrix (El-Kassaby 1991) was carried out through NTSYS-PC version 2.1 software (Rohlf 2000). This type of survey helps to identify the genetic variables (allele frequencies) with greater variability.

## Results

### *Genetic variation within Tuscan populations*

The statistical analysis of data was performed on 5 out 6 analyzed isozyme systems, since the LAP system did not show clear banding patterns with unreadable allele variants.

The number of identified *loci* and the allele distribution observed in our study were similar to the results reported in Leinemann and Bendixen (*op. cit.*) and Bendixen (*op. cit.*). In general, the observed banding patterns showed Mendelian inheritance, according to Leinemann and Bendixen (*op. cit.*) for *A. campestre*, Konnert *et al.* (*op. cit.*) and Belletti *et al.* (*op. cit.*) for *A. pseudoplatanus*, Rusanen *et al.* (*op. cit.*) for *A. platanoides*, Iddrisu and Ritland (*op. cit.*) for *A. macrophillum.*

As for the studied systems, 11 *loci* were identified: 7 were polymorphic (PGI-2; PGM-2; PGM-3; ADH-2; IDH-1; GOT-2; GOT-3) and 4 monomorphic (PGI-1; PGM-1; ADH-1; GOT-1), with a total of 21 alleles. For the system IDH, only a single *locus* with 2 polymorphic alleles was detected. The slower variant, *b* labelled, was present in only three (Ca' de Cìo, Dagnano, Chiana) of the four populations in Tuscany, with a frequency 5%; this *b variant* resulted rare in our samples.

**Tab. 3** - Allele frequencies for isozyme systems analyzed in field maple populations (N = size of the population).
*Frequenze alleliche dei sistemi enzimatici analizzati nelle popolazioni di acero campestre (N = dimensione della popolazione).*

| | | Population | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Locus*** | **Allele** | **Ca' de Cìo (Ar)** | **Dagnano (Ar)** | **Pieve (Ar)** | **Chiana (Ar)** | **Cerreta (Sa)** | **Baia (Ce)** | **Vesolo (Sa)** |
| **PGI-1** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **Monomorphic** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.00 |
| **PGI-2** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| | **B** | .211 | .091 | .167 | .250 | .300 | .214 | .094 |
| | **C** | .000 | .000 | .000 | .000 | .000 | .000 | .000 |
| | **D** | .395 | .591 | .833 | .575 | .633 | .738 | .875 |
| | **E** | .395 | .318 | .000 | .175 | .067 | .048 | .031 |
| **PGM-1** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **Monomorphic** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **PGM-2** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .579 | .455 | .556 | .550 | .500 | .333 | .438 |
| | **B** | .421 | .545 | .444 | .450 | .500 | .667 | .563 |
| **PGM-3** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .579 | .591 | .611 | .675 | .467 | .690 | .250 |
| | **B** | .105 | .091 | .111 | .100 | .100 | .024 | .000 |
| | **C** | .316 | .318 | .278 | .225 | .433 | .286 | .750 |
| **ADH-1** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **Monomorphic** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **ADH-2** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .184 | .273 | .389 | .075 | .367 | .214 | .344 |
| | **B** | .684 | .545 | .556 | .600 | .433 | .595 | .625 |
| | **C** | .132 | .182 | .056 | .325 | .200 | .190 | .031 |
| **IDH-1** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .947 | .955 | 1.000 | .975 | 1.000 | 1.000 | 1.000 |
| | **B** | .053 | .045 | .000 | .025 | .000 | .000 | .000 |
| **GOT-1** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **Monomorphic** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| **GOT-2** | **N** | 18 | 11 | 8 | 19 | 15 | 21 | 15 |
| | **A** | .056 | .000 | .000 | .053 | .100 | .143 | .033 |
| | **B** | .750 | .682 | .625 | .658 | .867 | .667 | .767 |
| | **C** | .194 | .318 | .375 | .289 | .033 | .190 | .200 |
| **GOT-3** | **N** | 19 | 11 | 9 | 20 | 15 | 21 | 16 |
| | **A** | .026 | .000 | .000 | .000 | .033 | .048 | .031 |
| | **B** | .974 | 1.000 | .944 | 1.000 | .967 | .952 | .969 |
| | **C** | .000 | .000 | .056 | .000 | .000 | .000 | .000 |

Allele distribution and frequency in polymorphic *loci* was similar in the 4 analyzed populations (Table 3), with exception of *locus* GOT-3. Indeed, the allele GOT-3*a* was present only in population Ca' de Cìo, while only in Pieve population GOT-3*c* was detected. The allele GOT-3*b* resulted monomorphic in Dagnano and Chiana, whereas the allele GOT-2*a* was present only in Ca' de Cìo and Chiana with a rare frequency (0.056 and 0.053, respectively).

The mean number of alleles per *locus* (A) was 1.85 and ranged from 1.7 to 2.0 (Pieve and Ca' de Cìo, respectively), while mean effective number of allele per *locus* ($A_e$) was 1.49 (Table 4). In Pieve population allele PGI-2*e* was absent and *locus* IDH-1 was monomorphic. Moreover, the allele ADH-2*a* was quite rare (0.056). The mean value of percentage of polymorphic *loci* ($P_{5\%}$) was 50.0 % in Tuscan populations (Table 4). Ca' de Cìo and Pieve showed the highest value ($P_{5\%}$ = 54.5), while Dagnano and Chiana showed the lowest value ($P_{5\%}$ = 45.5). The mean observed heterozygosity ($H_o$) was 0.205 ranging from 0.178 (Chiana) to 0.264 (Dagnano); the mean expected heterozygosity ($H_e$) was 0.247, from 0.233 (Pieve) to 0.257 (Dagnano).

**Tab. 4** – Indices of genetic variability for field maple populations estimated for all analysed *loci*. N/L: mean sample size per *locus*; A: mean number of alleles per *locus*; $A_e$: effective number of allele per *locus*; $P_{5\%}$: percentage of polymorphic *loci*; $H_o$: mean observed heterozygosity; $H_e$: mean expected heterozygosity (Nei 1978); F: mean fixation index per population or inbreeding coefficient (Wrigth 1978). Standard error in brackets (*significance level for P<0.05).
*Indici di variabilità genetica stimati per le popolazioni di acero campestre per tutti i* loci *analizzati. N/L: dimensione media del campione per* locus*; A: numero medio di alleli per* locus*; $A_e$: numero effettivo di alleli per* locus*; $P_{5\%}$: percentuale di* loci *polimorfici; $H_o$: eterozigosi media osservata; $H_e$: eterozigosi media attesa (Nei 1978); F: indice di fissazione medio per popolazione o coefficiente di inincrocio (Wrigth 1978). Errore standard tra parentesi (*livello di significatività per P<0.05).*

| Population | N/L | A | $A_e$ | $P_{5\%}$ | $H_o$ | $H_e$ | F |
|---|---|---|---|---|---|---|---|
| 1. Ca' de Cìo (Ar) | 18.9<br>( .1) | 2.0<br>( .3) | 1.52<br>( .103) | 54.5 | .192<br>( .064) | .253<br>( .081) | .241* |
| 2. Dagnano (Ar) | 11.0<br>( .0) | 1.8<br>( .3) | 1.52<br>( .128) | 45.5 | .264<br>( .094) | .257<br>( .085) | -.027 |
| 3. Pieve (Ar) | 8.9<br>( .1) | 1.7<br>( .2) | 1.42<br>( .118) | 54.5 | .184<br>( .066) | .233<br>( .078) | .210 |
| 4. Chiana (Ar) | 19.9<br>( .1) | 1.9<br>( .3) | 1.48<br>( .089) | 45.5 | .178<br>( .061) | .244<br>( .082) | .270* |
| **Tuscan population mean** | | **1.85**<br>**(.06)** | **1.49**<br>**( .024)** | **50.0** | **.205**<br>**(.020)** | **.247**<br>**(.005)** | **.174** |
| 5. Cerreta (Sa) | 15.0<br>( .0) | 1.9<br>( .3) | 1.51<br>( .121) | 45.5 | .218<br>( .081) | .238<br>( .084) | .084 |
| 6. Baia (Ce) | 21.0<br>( .0) | 1.9<br>( .3) | 1.42<br>( .076) | 45.5 | .173<br>( .056) | .228<br>( .075) | .241* |
| 7. Vesolo (Sa) | 15.9<br>( .1) | 1.8<br>( .3) | 1.31<br>( .069) | 45.5 | .132<br>( .046) | .189<br>( .066) | .302* |
| **Campania population mean** | | **1.87**<br>**(.03)** | **1.41**<br>**( .058)** | **45.5** | **.174**<br>**(.025)** | **.218**<br>**(.015)** | **.209** |
| **General mean** | | **1.86**<br>**(.04)** | **1.45**<br>**( .029)** | **48.07** | **.192**<br>**(.015)** | **.235**<br>**(.008)** | **.189** |

**Tab. 5** - Mean fixation index (F) assessed for each population and polymorphic *locus* (* P ≤ 0.05; ** P ≤ 0.01).
*Indice di fissazione medio (F) stimato per ciascuna popolazione e* locus *polimorfico (* P ≤ 0,05; ** P ≤ 0,01).*

| | | | | ***Locus*** | | | |
|---|---|---|---|---|---|---|---|
| **Population** | **PGI-2** | **PGM-2** | **PGM-3** | **ADH-2** | **IDH-1** | **GOT-2** | **GOT-3** |
| Ca' de Cìo (Ar) | .183 | .136 | .240 | .124 | -.056 | .580** | -.027 |
| Dagnano (Ar) | -.344 | -.100 | -.008 | -.222 | -.048 | .371 | - |
| Pieve (Ar) | -.200 | -.350 | .379 | .379 | - | .467 | -.059 |
| Chiana (Ar) | .306 | -.010 | .173 | .338 | -.026 | .452** | - |
| Baia (Ce) | .298 | .143 | .244 | .239 | - | .236* | -.050 |
| Cerreta (Sa) | .075 | -.200 | -.027* | .164 | - | .439* | -.034 |
| Vesolo (Sa) | -.113 | .238 | .000 | .618* | - | .461 | -.032 |

**Tab. 6** - Mean F-statistics assessed over all *loci* and populations analysed.
*F-statistici medi stimati per tutti i* loci *e tutte le popolazioni analizzate.*

| Locus | FIS | FIT | FST |
|---|---|---|---|
| PGI-2 | .026 | .113 | .090 |
| PGM-2 | -.082 | -.072 | .009 |
| PGM-3 | .197 | .201 | .005 |
| ADH-2 | .145 | .184 | .046 |
| IDH-1 | -.046 | -.032 | .014 |
| GOT-2 | .465 | .473 | .016 |
| GOT-3 | -.048 | -.016 | .031 |
| **Mean** | **.137** | **.166** | **.034** |

As $H_e$ was generally higher than $H_o$ value, the mean inbreeding coefficient (Table 4) resulted positive (F = 0.174). Dagnano only showed a negative F value (-0.027) near to the equilibrium. The significant departures from the Hardy-Weinberg equilibrium per *locus* were probably due to an excess of homozygotes at *locus* Got-2 in populations Ca' de Cìo and Chiana (Table 5).

Mean value of F-statistic indices, assessed for all *loci* and Tuscan populations (Table 6), showed that the 83% of genetic variability was found within populations (mean $F_{IS}$ = 0.137), whereas $F_{ST}$ (genetic variability among populations) was only 34%. The $F_{IT}$ mean value (0.166) highlights the lack of heterozygotes in the overall population. GOT-2 (0.473), PGM-3 (0.201), ADH-2 (0.184) were *loci* that mostly contributed to achieve this result.

### *Genetic variation within reference populations*

The same isozyme systems were analysed for 3 field maple populations selected in Campania and used as reference.

Comparison between distribution and frequency of alleles (Table 3) revealed that in Campania populations the system IDH-1 was monomorphic for allele *a* and the allele PGI-2*e* had a lesser frequency compared to the Tuscan populations (in Baia-Caserta and Vesolo-Salerno, PGI-2*e* was rare). At *locus* GOT-2 the allele *a* was rare only in Vesolo, while in the Tuscan populations it was rare (Ca' de Cìo and Chiana) or null (Dagnano and Pieve). The allele GOT-3*a* was rare but present in the three reference populations, while it was detected in the Tuscan population Ca' de Cìo only (Table 3). Finally, in Vesolo allele PGM-2*b* was probably null.

The mean number of allele per *locus* (A) was 1.87 in Campania populations and ranged from 1.8 (Vesolo) and 1.9 (Baia and Cerreta; Table 4). Mean value of $P_{5\%}$ was lower in the reference system (mean $P_{5\%}$ Campania = 45.5; mean $P_{5\%}$ Tuscany = 50.0). In the reference system mean $H_o$ value was 0.174, while mean $H_e$ was 0.218. The inbreeding coefficient was always positive (excess of homozygotes), but the population of Cerreta (F = 0.084) could be considered close to Hardy-Weinberg equilibrium. Instead, Vesolo was the population with the highest value of F (0.302) among all those analyzed in this study (Table 4). Baia and Cerreta deviated significantly from panmittic equilibrium at *locus* GOT-2 for homozygote excess, as well as Vesolo deviated from the equilibrium at locus ADH-2 for the same reason (Table 5). F was significantly negative at *locus* PGM-3 in Cerreta population (excess of heterozygotes) (Table 5).

**Tab. 8** - Observed (A) and expected ($A_e$) number of alleles, observed ($H_o$) and expected ($H_e$) heterozygosity estimated for polymorphic *loci* in all analyzed populations.
*Numero di alleli osservato (A) ed atteso ($A_e$), eterozigosi osservata ($H_o$) e attesa ($H_e$) stimate per i* loci *polimorfici in tutte le popolazioni.*

| *Locus* | Number of alleles | | $H_o$ | $H_e$ | |
|---|---|---|---|---|---|
| | A | $A_e$ | | Levene's | Nei's |
| PGI-2 | 3 | 2.07 | 0.4144 | 0.5189 | 0.5165 |
| PGM-2 | 2 | 2.00 | 0.4775 | 0.5016 | 0.4994 |
| PGM-3 | 3 | 2.20 | 0.4234 | 0.5489 | 0.5464 |
| ADH-2 | 3 | 2.32 | 0.4144 | 0.5712 | 0.5686 |
| IDH-1 | 2 | 1.04 | 0.0360 | 0.0355 | 0.0354 |
| GOT-2 | 3 | 1.73 | 0.2342 | 0.4225 | 0.4206 |
| GOT-3 | 3 | 1.06 | 0.0541 | 0.0530 | 0.0528 |
| **Mean** | **2.71** | **1.77** | **0.2934** | **0.3788** | **0.3771** |
| **SD** | **0.4880** | **0.5274** | **0.1858** | **0.2333** | **0.2322** |

### *Genetic structure of studied populations*

For the total sample, the neutrality of analysed markers - tested by Ewens-Watterson test for neutrality - suggested that all polymorphic *loci* were neutral at 0.01 level (Table 7) indicating no effect of selection on allele distribution. $\chi^2$ and $G^2$ (likelihood ratio) tests showed that all polymorphic *loci*, except for GOT-2, deviated from Hardy-Weinberg equilibrium (probability: $\chi^2/G^2$ >0.05). Furthermore, only IDH-1 and GOT-3 *loci* showed a little higher observed heterozygosity than expected (Table 8).

Wright's F, found significantly different from 0 (P<0.05) in Ca' de Cìo and Chiana (Tuscany) as well as in Baia and Vesolo (Campania), indicanted a deficiency of heterozygotes (Table 4). Global test for Hardy-Weinberg equilibrium (Markov chain parameters: 10,000 dememorization; 100 batches, 5,000 iteration per batch) was non significant (P<0.001) due to heterozygotes excess, both for populations and *loci*. On the contrary, deviation was significant (P<0.001) when deficit of hererozygotes was considered.

Linkage disequilibrium test for single populations (calculated using 1000 similated samples) showed

**Tab. 7** - Chi-square and G square probabilities (95% confidence level), mean F (mean homozigosity value expected under neutrality hypothesis), standard error (s.e.), lower (L95) and upper (U95) confidence limits of expected F values across 7 polymorphic *loci* in field maple populations. (* deviation from Hardy-Weinberg equilibrium).
*Livelli di probabilità relativi al Chi quadrato e al G quadrato (95%), F medio (omozigosi media attesa sotto ipotesi di neutralità), errore standard (s.e.), limiti degli intervalli di confidenza (minore: L95; maggiore U95) dei valori attesi di F nei 7* loci *polimorfici esaminati nelle 7 popolazioni di acero campestre (* deviazione dall'equilibro di Hardy-Weinberg).*

| | Hardy-Weinberg equilibrium test | | | Ewens-Watterson neutrality test | | | |
|---|---|---|---|---|---|---|---|
| *Locus* | DF | Probability $\chi^2$ | Probability $G^2$ | Mean F | s.e. | L95 | U95 |
| PGI-2 | 3* | 0.011499 | 0.025103 | 0.7169 | 0.034 | 0.3824 | 0.9733 |
| PGM-2 | 1* | 0.610626 | 0.610597 | 0.8320 | 0.028 | 0.5033 | 0.9910 |
| PGM-3 | 3* | 0.007027 | 0.014026 | 0.7075 | 0.035 | 0.3826 | 0.9821 |
| ADH-2 | 3* | 0.000104 | 0.000092 | 0.7081 | 0.036 | 0.3874 | 0.9821 |
| IDH-1 | 1* | 0.867336 | 0.814499 | 0.8297 | 0.028 | 0.5033 | 0.9910 |
| GOT-2 | 3 | 0.000000 | 0.000000 | 0.7072 | 0.037 | 0.3662 | 0.9821 |
| GOT-3 | 3* | 0.995068 | 0.986790 | 0.7000 | 0.038 | 0.3606 | 0.9821 |

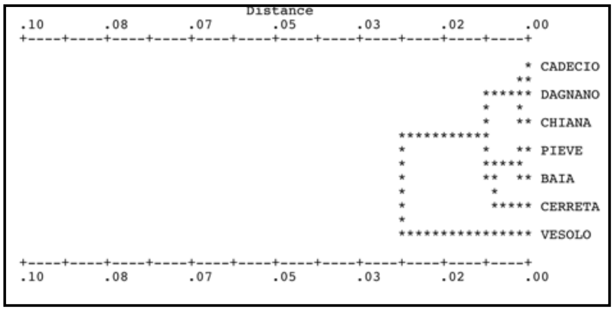
| Probability $\chi^2$ | Probability $G^2$ | $\chi^2/G^2$(>0.05) |
|---|---|---|
| 0.011499 | 0.0251030 | 0.458073 |
| 0.610626 | 0.610597 | 1.000047 |
| 0.007027 | 0.014026 | 0.500998 |
| 0.000104 | 0.000092 | 1.130435 |
| 0.867336 | 0.814499 | 1.064871 |
| 0 | 0 | 0.000000 |
| 0.995068 | 0.98679 | 1.008389 |

**Tab. 9** - Mean values of F statistics (Weir and Cockerham 1984) and genetic differentiation (Nei 1987; Hartl and Clark 1989) assessed for all *loci* and analysed populations of field maple.
*Valori medi degli F statistici (Weir and Cockerham 1984) e di differenziazione genetica (Nei 1987; Hartl and Clark 1989) stimati per tutti i* loci *e per le popolazioni analizzate.*

| *Locus* | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | $H_T$ | $H_S$ | $D_{ST}$ | $G_{ST}$ |
|---|---|---|---|---|---|---|---|
| PGI-2 | .059 | .151 | .098 | .511 | .471 | .040 | .078 |
| PGM-2 | -.024 | .002 | .025 | .502 | .505 | -.003 | -.005 |
| PGM-3 | .145 | .220 | .088 | .559 | .524 | .036 | .064 |
| ADH-2 | .222 | .256 | .043 | .576 | .572 | .004 | .007 |
| IDH-1 | -.046 | -.018 | .027 | .035 | .035 | -0.00 | -.002 |
| GOT-2 | .423 | .448 | .044 | .435 | .435 | -.001 | -.002 |
| GOT-3 | -.044 | -.023 | .020 | .054 | .055 | -.001 | -.011 |
| **Mean** | **.153** | **.203** | **.059** | **.243** | **.236** | **.007** | **.028** |

**Tab. 10** - Genetic distances among the studied populations assessed on all analysed *loci* (above diagonal: Nei (1978) unbiased genetic distance; below diagonal: Nei (1972) genetic distance).
*Distanze genetiche stimate tra le popolazioni studiate per i* loci *esaminati sopra la diagonale: Nei (1978); sotto la diagonale: Nei (1972).*

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. Ca' de Cìo (Ar) | ***** | .000 | .016 | .002 | .012 | .017 | .037 |
| 2. Dagnano (Ar) | .009 | ***** | .000 | .000 | .005 | .001 | .019 |
| 3. Pieve (Ar) | .029 | .014 | ***** | .005 | .006 | .002 | .014 |
| 4. Chiana (Ar) | .011 | .009 | .018 | ***** | .012 | .005 | .039 |
| 5. Cerreta (Sa) | .022 | .018 | .020 | .021 | ***** | .007 | .012 |
| 6. Baia (Ce) | .025 | .012 | .014 | .012 | .016 | ***** | .023 |
| 7. Vesolo (Sa) | .045 | .031 | .027 | .047 | .021 | .030 | ***** |



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                           Distance
.10       .08       .07       .05       .03       .02       .00
+----+----+----+----+----+----+----+----+----+----+----+----+

                                                            * CADECIO
                                                           **
                                                       ****** DAGNANO
                                                       *   *
                                                       *   ** CHIANA
                                             ***********
                                             *         *   ** PIEVE
                                             *         *****
                                             *         **  ** BAIA
                                             *          *
                                             *          ***** CERRETA
                                             *
                                             **************** VESOLO

+----+----+----+----+----+----+----+----+----+----+----+----+
.10       .08       .07       .05       .03       .02       .00
```

**Fig. 4** - UPGMA dendrogram (Nei 1978).
*Dendrogramma delle distanze genetiche UPGMA (Nei 1978).*

two numbers of significant (P<0.05; $\chi^2$ test with one degree of freedom): ADH-2*c* and GOT3*a*, ADH-2*c* and GOT-3*b*. When the test was made in pairs of *loci* across the seven populations analysed, it was non significant (P>0.05; Markov chain parameters: 10,000 dememorization; 100 batches, 5,000 iteration per batch), except in 6 out of 111 pairs (about 5.5%) which showed non-random associations between some pairs. After Fisher's test, genotypic linkage disequilibrium was significant (P<0.05) only between one *loci* pair (PGM-3 and ADH-2). Genic differentiation (Markov chain parameters: 10.000 dememorization; 100 batches, 5.000 iteration per batch) across all *loci* of all populations (Fisher's exact test P≤ 0.001) was significative only for PGI-2. When population pairs were considered, allele frequencies across all *loci* was not significantly different between populations (Fisher's exact test P≤ 0.001), except for one population pair (Chiana and Vesolo).

Even when F-statistics were calculated on all populations analyzed, approximately 94% of the total variability ($F_{ST}$ = 0.059 and $F_{IT}$ = 0.203) was common to 7 groups. Consequently, according to Wright (*op. cit.*), it could be said that there was a moderate differentiation among populations and the most important factor of variability was the genetic diversity within populations ($F_{IS}$ = 0.153; Table 9). The mean values of genetic differentiation confirmed that the inter-population variability was only a small part of the total ($H_T$ = 0.243; $G_{ST}$ = 0.028). The *locus* IDH-1 was the one with less total variability, while PGI-2 ($G_{ST}$ = 0.078) and PGM-3 ($G_{ST}$ = 0.064) were the most effective in differentiating populations. The little proportion of genetic variation due to differences among populations may be caused by a high level of gene flow, as indicated both by the indirect method ($N_m$ = 4.017; Wright *op. cit.*) and by the private allele method ($N_m$ = 4.190; $N_m$ = 2.656 after correction for size; Slatkin *op. cit.*; Barton and Slatkin *op. cit.*).

The genetic variability mainly due to differences among individuals was also confirmed by the dendrogram obtained with the total samples (by NTSYS 2.1; Rohlf *op. cit.*); some individuals sampled in Tuscan populations were grouped, but most of them were distributed in other clusters with Campania trees.

Nei's genetic distance values among populations were low (Table 10). Among the populations used as reference system, Vesolo was the population with greater distance from Tuscan populations. The UPGMA dendrogram, based on Nei's genetic distances (*op. cit.*; Figure 4), had two main clusters: the first was homogenous from a geographical point of view, bringing together 3 out of 4 Tuscan populations (Ca' de Cìo, Dagnano, Chiana), while the second included 2 Campania populations (Baia and Cerreta) and 1 Tuscan population (Pieve). The population of Vesolo was the most genetically distinct population, since it was separated from the other populations and formed a single cluster.

The principal component analysis (Table 11), assessed on all analyzed populations, showed that the first six components explain 56% of the total variance, while the first three accounted the 32% only. The alleles with more discriminating value resulted ADH-2b, ADH-2c, PGM-3b, PGM-2b, GOT-2b and GOT-2c,

**Tab. 11** - Principal components analysis assessed on the matrix of 18 alleles in the 7 isoenzymatic polymorphic *loci*.
*Analisi delle Componenti principali stimate sulla matrice di 18 alleli in 7* loci *enzimatici polimorfici.*

| Component | Eigenvalue | Simple % | Cumulated % | Allele | Correlation coefficient |
|---|---|---|---|---|---|
| 1 | 2.065 | 12.145 | 12.145 | ADH-2b | 0.81 |
| 2 | 1.806 | 10.624 | 22.769 | ADH-2c | -0.71 |
| 3 | 1.664 | 9.789 | 32.558 | PGM-3b | 0.61 |
| 4 | 1.422 | 8.364 | 40.922 | PGM-2b | -0.60 |
| 5 | 1.355 | 7.971 | 48.893 | GOT-2b | 0.58 |
| 6 | 1.236 | 7.268 | 56.161 | GOT-2c | -0.58 |

respectively. The plot in Figure 5 also confirmed the separation of Tuscan populations, Ca' de Cìo, Dagnano and Chiana from those of Campania region. Vesolo resulted separated from the other groups.

## Discussion

### *Genetic variation and structure*

As regards the main genetic parameters, the results obtained in this study were comparable with those reported in literature for *A. campestre* (Bendixen *op. cit.*) and other species of genus *Acer*, characterized by different natural distribution and types of diffusion (Perry and Knowles 1989; Belletti and Monteleone *op. cit.*; Rusanen *et al. op. cit.*; Iddrisu and Ritland *op. cit.*; Belletti *et al. op. cit.*).

Mean number of alleles per *locus* was less (A = 2.09) than Bendixen's (*op. cit.*) for *A. campestre* (A = 3.15). However, this author did not include the monomorphic *loci* in his analysis. Even Belletti *et al. (op. cit.)* estimated a higher mean value (A = 2.78) for northern Italian populations of sycamore, even though the mean effective number of alleles per *locus* was 1.47 which was similar to the value estimated in this study (mean $A_e$ = 1.49 and 1.41 for Tuscan and Campania populations respectively, while mean $A_e$ = 1.45 for global sample). In other *Acer* species A ranged from 1.71 (bigleaf maple; Iddrisu and Ritland (*op. cit.*) to 2.03 (sugar maple; Foré *et al.* 1992b). Rusanen *et al.* (*op. cit.*) assessed mean A = 2.0 for *A. platanoides* in Northern Europe.

The mean percentage of polymorphic *loci* (48.7) is lower than the one indicated by Rusanen *et al.* (*op. cit.*) for Norway maple (54.5%) or by Iddrisu and Ritland *(op. cit.)* for *A. macrophyllum* ($P_{5\%}$ = 61.2%). On the contrary, $P_{5\%}$ was higher than the one found by Perry and Knowles (*op. cit.*) for *A. saccharum* ($P_{5\%}$ = 38.2%) and near the value estimated by Hamrick *et al.* (*op. cit.*) for woody Angiosperms (45.1). Foré *et al.* (1992a) obtained very high $P_{5\%}$ value (87.5) for *A. saccharum* in Canada and also Bendixen (*op. cit.*) for field maple in Germany, but they did not survey monomorphic *loci*. Perhaps our result was affected by the limited number of samples and populations and by the isozyme system analysed.

The mean heterozygosity (0.235) of this first group of analysed populations was relatively higher than the one estimated by Hamrick (1989) and Hamrick *et al.* (*op. cit.*) for woody species pollinated by wind or insect (mean H = 0.154 and H = 0.163 respectively), and by Foré *et al. op. cit.* ($H_e$ = 0.150), Perry and Knowles *op. cit.* ($H_e$ = 0.110), Young *et al.* (1993a; $H_e$ = 0.109) for *A. saccharum*, by Rusanen *et al. op. cit.* ($H_e$ = 0.128; $H_e$ = 0.132) for *A. platanoides*, by Iddrisu and Ritland *op. cit.* ($H_e$ = 0.152) for *A. macrophyllum*. However, Bendixen (*op. cit.*), still for field maple, obtained H value (0.287) higher than the value obtained in the present study. In addition, on 18 populations of *A. pseudoplatanus* selected in Northern Italy, Belletti and Monteleone *op. cit.* and Belletti *et al. op. cit.* obtained a mean value (H = 0.200) greater than Hamrick's (*op. cit.*) and Hamrick's *et al.* (*op. cit.*). In Italy similar values of H for species with sporadic spread, like wild cherry (Ducci and Santi 1996; Ducci and Proietti 1997; Proietti *et al.* 2006) and for social species (like genus *Quercus*, Belletti *et al.* 2005) were found. Higher values were estimated in Europe also for species with insect cross-pollination such as *Sorbus aucuparia* (Raspé and Jacquemart 1998) and *Sorbus torminalis* (Demesure *et al.* 2000) or for those with wind pollination such as *Fagus sylvatica* (Müller-Starch *et al.* 1992; Konnert 1995). Actually, in tree species with a wide and sporadic spreading range,

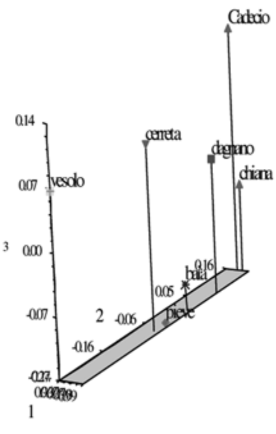

**Fig. 5** - Population division obtained from Principal Components Analysis.
*Divisione delle popolazioni ottenuta con l'Analisi delle Componenti Principali.*

these values could be determined by cross-pollination which promotes diversity among individuals in populations. *P. avium* and *Sorbus* sp., species similar to field maple from an ecological and biological point of view, could store more intraspecific diversity than species with other reproductive characteristics.

The excess of homozygotes was probably due to the size and type of sampled populations, which the artificial ones (lines on field edges) consist predominantly of seedlings collected by farmers from natural regeneration, originated by one or few parental trees and used as living props for grapevines; the natural materials come from relatively isolated populations consisting of few scattered trees. Therefore, their structure could be also affected by the "founder effect". It is worth that two natural populations used as local references, Dagnano in Tuscany and Cerreta in Campania, showed the lowest inbreeding coefficient. Indeed, those populations are located in forest and were sampled in areas with low anthropic influence.

Even the age of forest stand can affect the level of heterozygosity: the homozygotes tend to decrease with age, probably because of selection phenomena and adaptation to the environment (Rossi *et al.* 1996). Heterozygotes generally were considered more resistant to environmental stresses, then their percentage tends to increase in mature stands (Hertel *et al.* 1994). Consequently, the F value tends to decline.

F-statistics values indicated a low degree of differenziation in the studied populations ($F_{ST}$ = 0.059). However, the value of $F_{ST}$ found for *A. campestre* were similar to those reported for *A. saccharum* ($F_{ST}$ = 0.049, Young *et al.*, 1993b; $F_{ST}$ = 0.033, Perry and Knowles *op. cit.*), *A. macrophyllum* ($F_{ST}$ = 0.054, Iddrisu and Ritland *op. cit.*), *A. pseudoplatanus* ($F_{ST}$= 0.019, Belletti *et al. op. cit.*). Rusanen *et al.* (*op. cit.*) estimated an higher $F_{ST}$ for *A. platanoides* (0.099), but populations analyzed were selected in a much wider area (Germany, Poland, Denmark, Estonia, Norway, Sweden and Finland). This result was frequent in woody *Angiospermae* with wide and continuous range, long biological cycles and cross-pollination. All these factors favoured exchanges among populations across pollen and seeds and may explain why the individual variability was greater than the total variability (Loveness and Hamrick 1984). Because molecular and biochemical genetic markers are generally considered neutrals, a further study with non-neutral markers (as stress resistance, phenology, shape indices) could be useful to better highlight differentiation among populations. Non-neutral markers could identify the adaptative genetic diversity (Kremer *et al. op. cit.*) that has enabled populations to survive to different environmental conditions in the course of time. $F_{ST}$value was confirmed also by $G_{ST}$ (0.028) that was lower than the value estimated by Hamrick *et al.* (*op. cit.*) for outcrossing species with seeds dispersed by wind (0.084). Probably this result reflected the behavior of field maple which is considered a post-pioneer species able to occupy abandoned areas such as non cultivated fields or pastures. The *loci* PGI-2 and PGM3 ($G_{ST}$ = 0.078 and 0.064 respectively) were those with higher capacity of population differentiation.

The low genetic differentiation reflect also the estensive gene flow (allogamy and seeds dispersed by wind) and recent colonization. Indeed, both factors promote similarity among populations. Furthermore, in *A. campestre* $F_{ST}$ could also be influenced by human activities. Disturbances on habitats occupied by field maple did not reduce gene flow, as indicated both by indirect estimate and private allele method. Instead, the high value of $N_m$ could contrast the effects of genetic drift and/or small size of isolated population and could be useful to preserve structure in populations. High level of migration among populations was found in Italy for *A. pseudoplatanus* ($N_m$ = 4.39; Belletti *et al. op. cit.*) and in Canada for *A. macrophyllum* ($N_m$ = 4.10; Iddrisu and Ritland *op. cit.*). The latter result suggested that geografical distances did not limit gene flow thanks to continuity of natural range, cross-pollination and ability of pollen and seed dispersion. All these factors tend to homogenize genetic composition of populations and to reduce their differentiation.

The reduced genetic distances between Tuscan and Campania populations could be explained by distribution of *A. campestre* (wide and continuous natural range) and by the manipulation that it has undergone over the past 10 centuries which has encouraged gene flow along the Italian peninsula. The UPGMA dendrogram partially reflects the geographical distribution of the analysed populations clustering only 3 Tuscan populations, but grouping the fourth Tuscan population with Baia and Cerreta. The apparent lack of clear geographical structure in genetic variability was found in Italy also for wild cherry (Ducci and Proietti *op. cit.*).

In other *Acer* species, authors (Iddrisu and Ritland *op. cit.*; Foré *et al. op. cit.*; Rusanen *et al. op. cit.*)

found similar value of Nei's genetic distance and non-significant, or only few significant, correlations between genetic and geographical distance, although the analysed populations were selected in wider regions (*e.g.* Canada for *A. macrophyllum* and *A. saccharum* or northern Europe for *A. platanoides*).

***Management and conservation***

Conservation of genetic variability is a focal point not only for species at risk of extinction but for all species, because diversity confers evolutionary plasticity to individuals and maintains their adaptative potential to environmental changes (Palmberg-Lerche 1996; Namkoong 1998; Tessier Du Cros *et al.* 2000). Preserving a large gene pool is also essential for breeding and future genetic improvement.

Environmental simplification can be a factor of the genetic variability reduction, particularly in species like field maple whose populations are naturally formed by few individuals. In forest species, subject to greater environmental risk because of their long biological life cycles, good levels of variability (n/l, F and $P_{5\%}$) encourage adaptability to change due to biotic and abiotic factors.

The population of Pieve, that in recent years has suffered a gradual reduction of trees, could be an example of the above factors, as it was the group with the lowest mean number of alleles per *locus* in the populations analyzed. Despite this feature, Pieve was the only one in the 7 populations selected to have allele GOT-3*c*, even though with rare frequency. For this reason, a proper management of this population could be necessary to preserve this private allele.

Moreover, despite the limited differentiation among populations, there were differences in the frequency of some alleles between the Tuscan populations and those of reference. For example, in Campania populations the allele PGI-2*e* was rare and system IDH was monomorphic, while there was the opposite situation for GOT-2*a* and GOT-2*b* allele frequency in Tuscan populations. Thus, it is necessary to know the genetic diversity of single populations and regions in order to include more representative samples of genetic variation of species in conservation planning and take into account local adaptative variation as well. Moreover, genetic structure partly reflects the action of population genetic processes, *e.g.* selection and genetic drift.

As in other allozyme survey on wood species (Loveless and Hamrick *op. cit.*), the main component of genetic variability was $F_{IS}$ (intra-populations). Therefore, in *ex-situ* conservation programmes fewer populations should be sampled, but with a bigger number of individuals within them. The choice of populations could be done primarily taking into account allelic richness and heterozygosity because they reflect the ability of a species to cope with future changes. Genetic distance among populations was not very useful in this case because an evident geographical pattern was not present.

*In-situ* conservation is also important, because it allows the species to keep evolving together with the environment (dynamic conservation). Therefore, a sustainable land management aimed to preserve ecological, biological and cultural diversity could also promote the conservation of genetic resources.

It is useful to keep on studying the genetic structure of *A. campestre* to know the size and distribution of variability that is still available. Further surveys should take into account ecological data, altitude and adaptative traits, in order to better examine inter-population differentiation and better understand the capabilities of field maple to cope with global climate change.

## Acknowledgments

The authors wish to thank the anonymous reviewer for his helpful comments that improved the final paper.

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