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FORTE NOVELLO OLD-GROWTH FOREST

The study has been carried-out to compare the genetic diversity between old-growth beech forest and post-harvested naturally regenerated stands. The purpose was also the evaluation of the possible genetic impact of harvesting systems; these information are needed for the development sustainable silvicultural methods.

Key words: *Fagus sylvatica*; genetic diversity; old-growth forest; silvicultural methods.

Parole chiave: *Fagus sylvatica*; diversità genetica; bosco vetusto; trattamenti selvicolturali.

1. INTRODUCTION

The biodiversity is the biological variability comprising all the living organisms and the environment where they live. The genetic diversity is essential for the maintaining of high biodiversity levels, because the genetic diversity provides raw material for the adaptation, evolution and survival of species and individuals. In particular, high levels of genetic diversity provide the evaluative potential forest functionality in face of natural disturbances and of environmental changes (MULLER-STARCK *et al.*, 1992; RADDI *et al.*, 1994; RAJORA *et al.*, 2000).

The anthropogenic perturbances on forests date back to antiquity as result of deforestation, exploitation, habitat fragmentation and alteration, demographic change, pollution, and domestication. The impacts of these events on genetic diversity are much more difficult to quantify. However, it has been showed that, every human action, has, as consequence, ecological and genetic effects (LEDIG, 1992).

In this context, conserving the genetic diversity of native forest tree populations has special significance.

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Old-growth forests are considered important pools for species conservation because provide special habitats for an array of forest-dependent wildlife. Large and structured older forest tree populations may serve as reservoir of reproductive fitness and also may be as superior seed sources and gene pools avoiding the negative effect of inbreeding and genetic drift (RAJORA, 2003).

Although the documentation on old-growth forests in Mediterranean area is very poor (NILSSON *et al.*, 2003), during the last years, this theme has received an increasing attention (PIOVESAN *et al.*, 2003; PIOVESAN *et al.*, 2005; GIANNINI and SUSMEL, 2006). Because the fact that the Italian forest cover has been modified as result of human needs, only some little old-growth forest patches are found, prevalently located in remote mountain areas.

In the Massif of Gran Sasso a possible old-growth beech forest was present, not interested by man-managing harvests during the last 400 years, in consequence of administrative conflict between the Municipalities of Pietracamela and Fano Adriano (Teramo province) (GABBRIELLI, 2005).

The results of a study carried-out to compare the genetic diversity between old-growth beech forest and post-harvested naturally regenerated stands are reported. The purpose was also the evaluation of the possible genetic impact of harvesting systems; these informations are needed for the development sustainable silvicultural systems.

2. MATERIALS AND METHODS

2.1. *Beech forests and analysis of forest stand structures*

The study was conducted in an old-growth beech forest (Fonte Novello Forest) (stand A) and a managed pure beech forest (stand B) (2 km distant a part), located on the north-east slope of Gran Sasso Massif, in Venacquaro Creek Valley (1150-1250 m a.s.l. altitude, 42°30'N latitude, 13°29'E longitude).

The study was based on a total census of the individual within each experimental site of 0.64 and 0.27 hectare for A and B stands, respectively.

For the graphical characterisation of the plots, the data on plot dimension, tree positions, tree species, diameters breast height, tree heights, crown bases and parameters of the crown forms were collected. For the characterisation of the plot position, the plots have the form of polygons. Their dimensions can be characterised by the corners of the polygons. One of the corners is taken as corner number one (C1). The other corners were named anti-clockwise with C2, C3 etc. Additionally to the plot corners, the position of one point PN situated in the northern direction from corner C1 was

determined. The positions of corners C1, C2, C3 etc. and point PN was given by x- and y- co-ordinates in a Cartesian system of co-ordinates. The co-ordinates of C1, C2, C3 etc. and PN were measured in meters (m). The tree positions were described by the x- and y- co-ordinates in the Cartesian system of co-ordinates. The co-ordinates were measured in meters (m). For the social position of a tree, the trees of the plot were classified into tree classes based on their relative vigour according to Kraft-IUFO stem-crown classification (PIUSSI, 1994). The point at which diameter breast high was measured on the tree is defined as 1.3 m from the ground. On hills, this is taken as 1.3 m from the ground level on the uphill side. The diameter breast high was measured in centimetres (cm). The total height of the tree was defined as the height to the highest growing point of the tree. The total tree height was measured in meters (m). The Crown height was the vertical distance from the ground level to the lowest whorl of living branches. The crown height was measured in meters (m). The crown projection area was approximated by at least 4 and at most 8 periphery point of the projection of the crown onto the ground. These were given in polar co-ordinates regarding the tree position as the co-ordinate origin. The crown radius was the distance between the centre of the stem and the point on the periphery of the crown projection. The angle was determined anti-clockwise between the northern direction and the direction from the centre of the stem to the point on the crown periphery. The distances were measured in meters (m). The angles are measured in degree ($^{\circ}$) between 0° and 360° . The angles and the corresponding distances to the crown periphery was sorted ascend by the angle i.e. ANGLE1 was the smallest angle and CROWNR1 the corresponding distance.

The computer program Bestand for analysis and description of forest stand structures was used (Annett Degenhardt, Landesforstanstalt Eberswalde; DYNABEECH project: QLK5-CT-1999-01210). The crown projection map shows the vertical projections of the individual crowns. Measured crown radii in N ($4 \leq N \leq 8$) different directions from the base of the individual stems form the basis of this representation. The measured crown radii define the shape of the crown projection area. Following, the mathematical centre of the area can be computed. Then the crown radii and the corresponding angles of the measured directions from the stem is transformed into polar co-ordinates relating to the centre of the crown projection area. Linear interpolation of measured, neighbouring crown radii yields approximate crown radii to any crown angle. So the edge of the crown projection area was defined approximately. Beside the graphical representation of the crown projections, crown projection areas and crown eccentricities were computed for each tree. The crown projection area (CRP) was computed as the area (m^2) of the approximating polygon of n sides,

where $n=72$ crown edge points were taken into account. The crown eccentricity (CEC) is given as

$$CEC = \sum_{i=1}^N \frac{\varepsilon_i}{N}$$

with

$$\varepsilon_i = \frac{\sqrt{r_i^{*2} - r_{i-1}^{*2}}}{\max(r_i^*, r_{i-1}^*)}$$

The r_i^* denote the measured crown radii relating to the computed mathematical centre of the crown projection area (FREIST-DORR, 1992). For all parts of the stand mean values of these two quantities are listed in the graphics. Additionally, the shelter is given. It is computed as the percentage of the area which is covered by the crown projection on the total area of the stand. For better orientation the crown number can be inserted into the crown projection map. A further useful stand graphics is the three-dimensional representation, which can produce an impression of the real forest stand. Basing on tree co-ordinates, breast-height diameters, tree heights, crown radii and crown heights a three dimensional image of the structure in the stand was constructed. Additionally, the mean crown radius (MCR) and the crown surface area (CSA) were computed for each tree. The mean crown radius (m) is given by:

$$MCR = \sum_{i=1}^N r_i^*$$

where are crown radii relating to the centre of the crown area. The crown surface area (m^2) is computed as the area of a paraboloidal surface (DONG and KRAMER, 1985):

$$CSA = \frac{\pi \cdot MCR}{6 \cdot l^2} \cdot \left(\left(4 \cdot l^2 + MCR^2 \right)^{\frac{3}{2}} - MCR^3 \right)$$

For all parts of the stand mean values of these quantities are listed in the graphics.

2.2. Sampling and DNA extraction

For each stand, dormant buds were taken from about 100 adults tree and stored at $-20^\circ C$. Total DNA was isolated from dormant buds (50-100 mg as starting material) using the DNeasy plant kit (QiAgen, Germany) following the manufacturer specifications.

2.3. RAPD analysis

Amplification reactions were performed by using primers, reaction mixture and cycling as reported (PAFFETTI *et al.*, 1996). The amplification patterns were analyzed with a scanner densitometer (model GDS2000; Ultra-Violet Products, Ltd., Cambridge, United Kingdom).

2.4. Data analysis

AMOVA of the amplification products was performed, and the vector of presence and absence of RAPD markers (1 indicated that a band was present on a gel, and 0 indicated that a band was not present) for each individual to compute the genetic distance for each pair of individuals was constructed. The measurement used was the Euclidean metric measurement (E) of EXCOFFIER *et al.* 1992 as defined by HUFF *et al.* 1993, as follows: $E = \epsilon^2_{xy} = n(1 - 2n_{xy}/n)$, where $2n_{xy}$ is the number of markers shared by two individuals and n is the total number of polymorphic sites. We used the AMOVA procedure (EXCOFFIER *et al.* 1992) to estimate the variance components of RAPD patterns, and to partition the variation among stands and within stands. All analyses were performed with the ARLEQUIN ver. 2.000 program provided by Excoffier (<http://anthro.unige.ch/arlequin>).

Spatial autocorrelation analysis was done to test whether the observed value of a nominal, ordinal or interval variable at one locality or individual classes is independent of values of the variable at neighbouring localities or individual classes (SOKAL and ODEN, 1978). The program Spatial Genetic Software (SGS) (<http://kourou.cirad.fr/genetique/software.html>, DEGEN, 2000; DEGEN *et al.*, 2001) provides a tool to analyse small and large scale genetic and phenotypic structures. RAPD data for each individual are transformed into a matrix of 1's and 0's, with 1 indicating presence of a certain fragment and 0 indicating its absence. The genetic dissimilarity [D_{ij}] between the binary vectors of two individuals, i and j , is computed, using the Tanimoto distance (DEICHSEL and TRAMPISCH, 1985):

$$0 \leq D_{ij} = 1 - [v_{ij} / (v_{ij} + y_i + y_j)] \leq 1$$

where v_{ij} represents the number of common fragments of individual i and j , the numbers of fragments which exist only in individuals i or j , respectively, are given by y_i and y_j . The overall mean of genetic distances between all individuals serves as a reference for random spatial structure. A permutation procedure using Monte-Carlo simulations are applied to test significant deviation from spatial random distribution of each calculated measure (MANLY, 1997). Each permutation consists of a random redistribution of genetic or phenotypic data over the spatial co-ordinates of the sampled points. For each of the spatial distance classes, observed values were

compared with the distribution obtained after N permutations. Then a X % confidence interval for the parameters is constructed as the interval from the CI 1 to the CI 2 ordered permutation estimates.

3. RESULTS AND DISCUSSION

Tree crown structure and tree crown planar view are reported for the two experimental plots (Figures 1a,b and 2a,b). The structure of forest cover (stand A) is dominated by the biostatic phase with the presence of few natural gaps derived from ancient trees coming down in which seedlings and saplings were present with "high herbes" and decay wood. The stand B was even-aged managed forest cover, with mean age of 120 years, derived from a coppice conversion to high forest by regular thinning finalized to harvest the smaller stems. Six years before the start of the study a seeding cutting was applied. The natural regeneration (seedlings) was localized at the centre of few lighting up gaps. The diameter distribution and the relationship among the diameter and height stems subdivided according to IUFRO's classification, are illustrated on Figures 3 and 4. The diameter tree distribution for the stand A, appears irregular and multi-modal, whereas a classic normal distribution is that of the stand B. The differences between the unmanaged (stand A) and managed (stand B) stands, are very evident (Table 1). The mean value of basal area of old-growth stand is about twice as much as that of the managed stand where, more than 2.5 time, the density (trees per hectare) was present.

Considering that the aim of the study is to evaluate the old-growth forest as resource of gene pool, a molecular genetic analysis by RAPD markers is carried out on the stand A and on stand B. The analyses of the data, conducted by AMOVA, indicated that the genetic difference between stand A and stand B was of the 5.5% ($p = 0.05\%$). This is also confirmed by the genetic difference among the individuals present in the stand A and in the stand B, respectively, 23.2 and 21.2 ($p = 0.001\%$), indicating that the individuals present in the stand A are more genetically different among them. As expected, the genetic data confirmed that the old growth forest of beech (stand A) present an higher genetic diversity respect to the managed beech stand B.

The spatial genetic structure of the stands has been conducted by SGS as reported in the materials and methods. The mean of the genetics distance for each stand has been calculated for 25 distance classes taking in consideration to have for each distance class 30 pairs of individuals. The spatial autocorrelation indicates a positive significant correlation at the distance class 1 and 10 for stand A, and at the distance classes 1, 5 and 11 for stand B. In general, the individuals tends to be randomly distributed in the space (Figures 5 and 6).

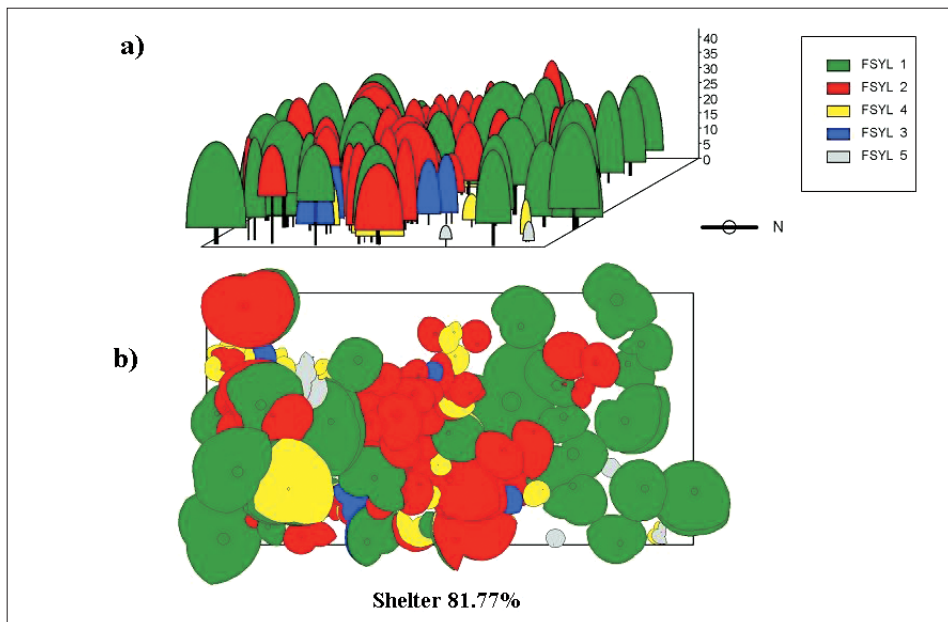


Figure 1 – Three-dimensional representational (a) and the crow projection map (b) of the stand A. In the box, FYSL is meaning *F. sylvatica*, and different colour indicates the social position of a tree according to Kraft-IUFO.

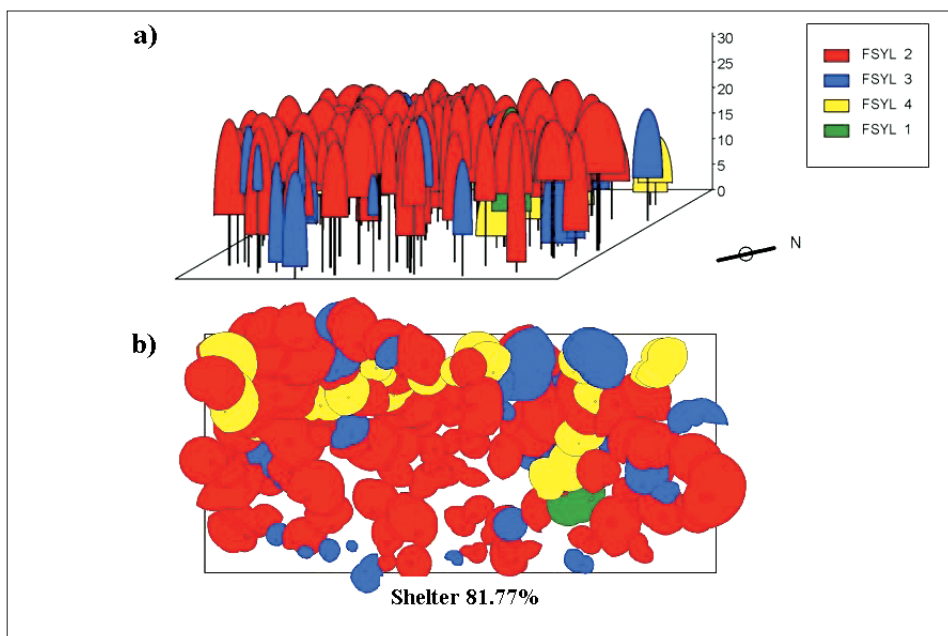


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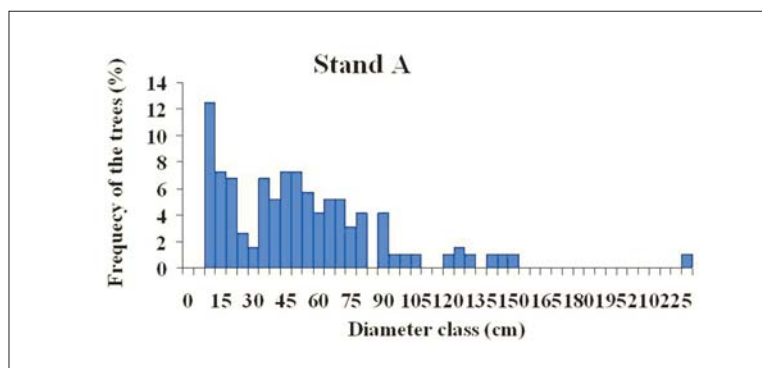


Figure 3 – The diameter class distribution of the trees present in the stand A.

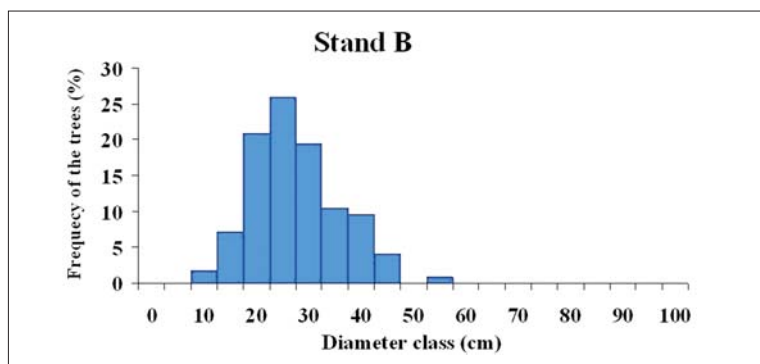


Figure 4 – The diameter class distribution of the trees present in the stand B.

Table 1 – Values of dendrometric characteristics of the stands A and B.

Stand	Density (Number of trees / ha)	Diameter (cm)			Basal area (m ² /ha)	Height of stem (m)			Age (years)		
		Min	Mean	Max		Min	Mean	Max	Min	Mean	Max
A	185	7.9	51.6	229.3	58.0	6.12	25.9	42.39	85	207	448
B	496	11.0	26.5	57.0	30.25	12.9	22.6	30.68	49	85	146

The frequency of all the RAPD markers (in total 87) was determined in each stand. In general, the RAPD markers showing a frequency ranging from 100% to 90% (red) in stand A maintain the same frequency in the stand B (figures 7 and 8). The markers presenting and intermediate frequency in the stand A maintain almost the same frequency in the stand B. On the contrary, those RAPD markers with low frequency (10%) (azure) or

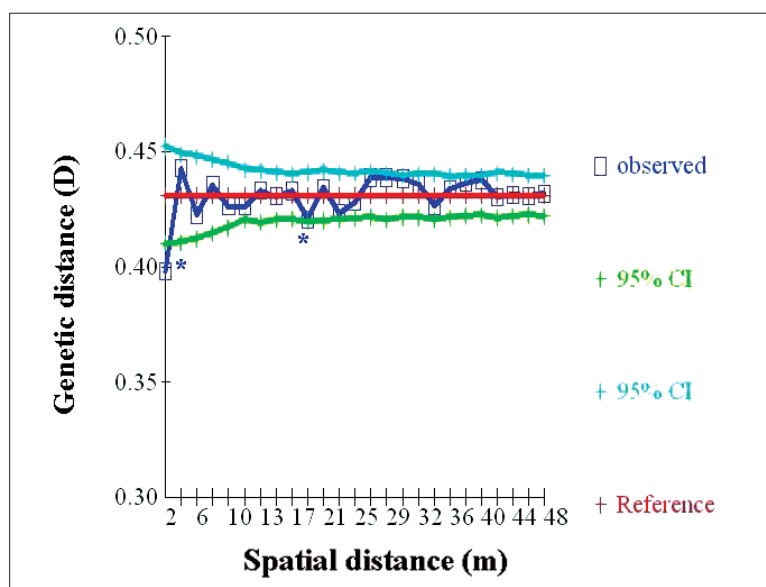


Figure 5 – Correlogram of stand A with a distigram calculated for RAPD data using the Tanimoto distance (D); “CI” = limit of the confidence interval; * significant D_k values estimated with 1000 permutations.

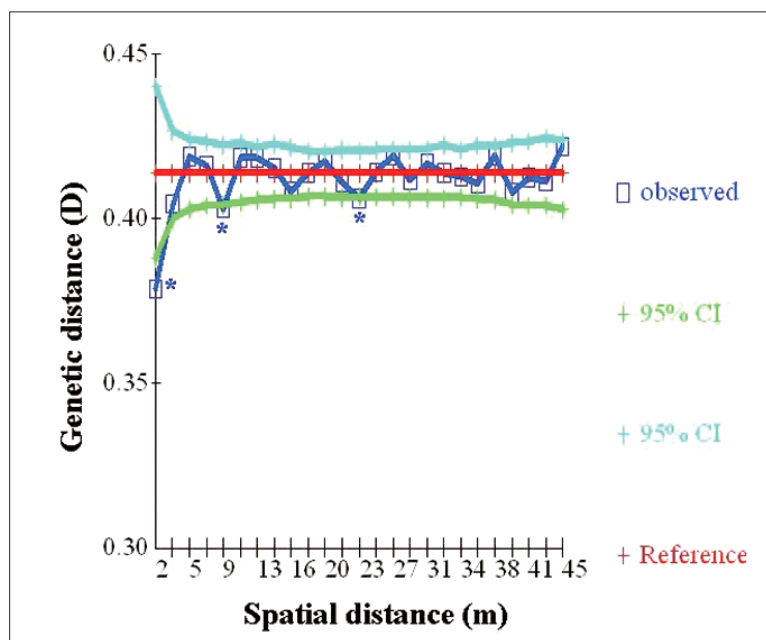


Figure 6 – Correlogram of stand B with a distigram calculated for RAPD data using the Tanimoto distance (D); “CI” = limit of the confidence interval; * significant D_k values estimated with 1000 permutations.

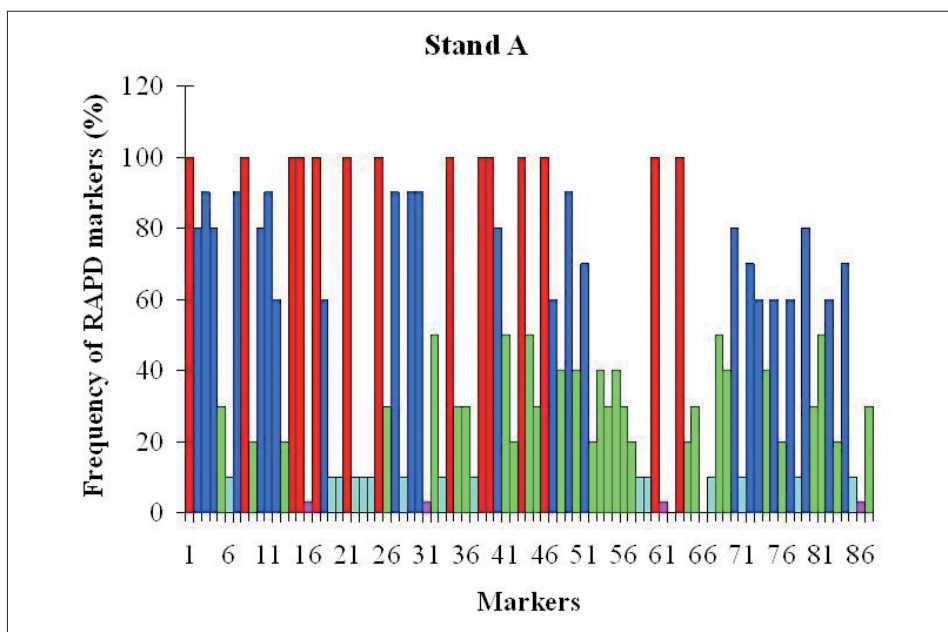


Figure 7 – Frequency of RAPD markers in the stand A. The colour indicates a frequency of: 100% (red); 90%-60% (blue), 50%-20% (green), 10% (azure), 3% (violet).

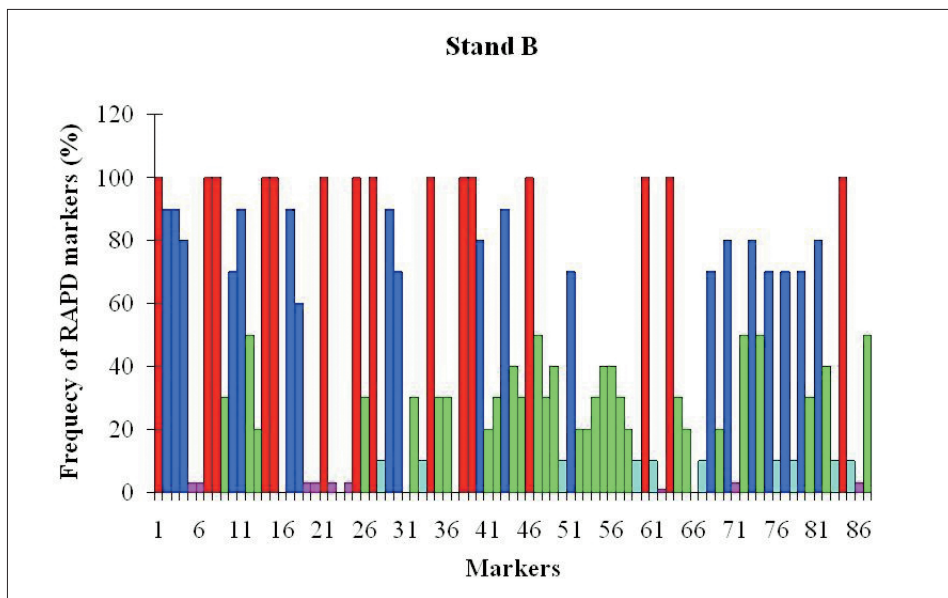


Figure 8 – Frequency of RAPD markers in the stand B. The colour indicates a frequency of: 100% (red); 90%-60% (blue), 50%-20% (green), 10% (azure), 3% (violet).

rare (3%) (violet) in the stand A tend to become rare or to disappear in the stand B, respectively (Figures 7 and 8; Table 2). This analysis shows that 13 RAPD markers present in the stand A become rare or are lost in the stand B. This result could be a consequence of the silviculture treatment conducted in the stand B.

4. CONCLUSIONS

During last years an increased number of researchers in ecology and genetics has carried out studies on the rule and importance of the *Old-Growth Forests*. This interest is coming from the conclusions of the Summit UNCED held in Brazil in 1992 and during which has

been indicated that the protection of the natural forest in relation to the biodiversity and to the ecology was a priority.

A full agreement on the term *Old-Growth Forests* has not been easy to reach. In general, this term indicates those forest ecosystems characterised by the presence of: successional natural phases of very old plants of big dimension, of stumps and died wood on the soil, of a crown pluri-stratification, of canopy gaps and of natural regeneration (EVERETT *et al.*, 1994; KAUFMANN *et al.*, 1992). In effect, this thematic is in relation to the concept of “naturalist” which is a wide term including several aspects of different disciplines (aesthetic, spiritual, social, economic, cultural, historic, temporal, law regulation, and ecologic). In addition, it has to be considered if the human has to be considered as part of the “nature” when “the natural environment” is defined. The response to this last question depends from the personal perception and from the own conscience. The ecological literature considers the concept of naturalist as a process, an event or a system which is not influenced by the human activity (or at least the influence of the human is comparable to that of other organisms).

The theoretical concept of natural forest means “a forest not influenced by any human activities”. Nevertheless, this definition is still too wide considering that the forest ecosystems present an high variability and complexity and that deep relationships and interactions between men and forest have been established during the time.

Table 2 – Frequency of the RAPD markers with low frequency or rare of the stand A and which become rare or disappear in the stand B. The frequency is expressed in %, as the number of markers found respect to the total number of the markers.

Markers	RAPD markers frequency (%)	
	Stand	
	A	B
6	10	3
16	3	0
19	10	3
20	10	3
22	10	3
23	10	0
24	10	3
28	10	10
31	3	0
33	10	10
37	10	0
85	10	10
86	3	3

The work by Erik BUCHWALD, presented at the “*Second Expert Meeting on Harmonising Forest-related Definitions*” held in Rome in 2002 (FAO, 2002), has given an important contribution in defining the concept of “Old-Growth Forest”. A hierarchical scale has been considered in which the principal components are the concept of natural and the different levels of human impact. Using this scale, the “Old-Growth Forest” are collocated in a medium position between the “Near Virgin Forests” and the “Long Untouched Forests”, and, in addition to the characteristics indicated by EVERETT *et al.* (1994), a relevant rule is given to the presence of a series of successional of structural and descriptive phases of biomass repartition. Therefore, the presence of died wood has been considered as an indicator for the evaluation of the biodiversity and naturality of a forest system as it is an indicator of many microhabitat health and functionality of hundreds of living organisms (SPIES, 2004).

The main interest and importance is focalised on the presence of died wood and on its role in carbon cycle. Recent studies have indicated that forests, also 800 years old, are still able to accumulate carbon after the homeostasis phase (ZHOU *et al.*, 2006; LUYSSAERT *et al.*, 2008). This data are going to modify the concept based on the homeostatic equilibrium, the neutrality of the carbon balance, defined by CLEMENTS (1916) and also taken by ODUM (1969).

Although, it is impossible to identify natural forest in our territory, several “old forest” can be considered in a phase similar to the “Old-Growth Forests” presenting the oldest trees discovered up to now (PIOVESAN *et al.*, 2005; MARCHETTI and LOMBARDI, 2006).

The present paper can give a preliminary contribution to the knowledge on the genetic structure of one of the older beech forest in Italy, and which can be considered close to nature because it has not been interested by man-managing harvests during long time. The genetic diversity is within population, and therefore at individual level. The low spatial genetic structure can be ascribed to the density of the forest and to the high level of gene flow typical of this allogame species with low level of inbreeding (BUCCI *et al.*, 1999).

An additional contribution is given in the evaluation of the effect on forest utilisation performed by cutting, which can produce to a slow loss of genetic diversity in the man-managed stand.

From a general point of view it is important to confirm that the Old-growth Forests can be considered natural reserves of biodiversity and reservoirs of genetic diversity (RAJORA and MOSSELER, 2001; GIANNINI and SUSMEL, 2006). This is an important aspect because the “genetic pool” of the population should have to be preserved and considered as a priority in the

conservation strategy. In the contest of the global change, the preservation of the natural forest has to be considered very carefully considering that the human activities can determine non-reversible consequences on the potential adaptability of the trees.

RIASSUNTO

Il bosco vetusto di Fonte Novello

La ricerca è stata condotta al fine di comparare la diversità genetica tra una faggeta assimilabile ad un bosco vetusto ed un soprassuolo limitrofo della stessa specie antropizzato e interessato da interventi selvicolturali (tagli successivi uniformi). La valutazione del possibile impatto sulla diversità genetica del popolamento da parte del trattamento è informazione indispensabile per una corretta applicazione di una gestione selvicolturale ecosostenibile.

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