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GEOGRAPHIC DISTRIBUTION OF GENETIC VARIABILITY OF *FAGUS SYLVATICA* L. IN SOUTHERN ITALIAN POPULATIONS [1]

G. Emiliani [1], D. Paffetti [1], C. Vettori [2] & R. Giannini [1, 2]

[1] Department of Environmental and Forestry Technology and Sciences, University of Florence, Via S. Bonaventura 13,
I-50145, Florence, Italy
[2] Institute of Plant Genetics, Division of Florence – National Research Council, Via Madonna del Piano,
I-50019 Sesto Fiorentino, Florence, Italy
Corresponding author: Prof. Raffaello Giannnini, Institute of Plant Genetics, National Research Council,
via Madonna del Piano, I-50019 Sesto Fiorentino, Florence, Italy. Phone: +39-055 5225704, Fax : +39-055 5225729,
e-mail: raffaello.giannini@igv.cnr.it

ABSTRACT

The actual distribution of *Fagus sylvatica* L. in Southern Italy has been influenced by past events such as glaciations.

The aim of the present work is to study the Southern Italian populations by: (i) PCR/RFLP markers of chloroplast DNA (cpDNA) to determine the geographical distribution of haplotypes, and (ii) RAPD markers of the total genome to evaluate the inter- and intra-population genetic variability. Thirty populations have been sampled along the natural range of *F. sylvatica* in Southern Italy. The analysis of cpDNA data showed that in the south of Italy: (i) more than one refuge nucleus was present, and (ii) genetic variability is high both at inter and intra population level. This is corroborated by the molecular variance analysis of the RAPD markers. The genetic variability resulted higher within populations than among populations, resulting in the latter parameter being substantially higher than other reported in literature for European stands of beech.

Keywords: *Fagus sylvatica* L., PCR/RFLP, cpDNA haplotypes, RAPD markers

INTRODUCTION

Much is known about quaternary climatic history and its influence on European taxa. Paleoclimatology, geological, palynological (HUNTLEY & BIRKS 1983), and genetic analysis, (DEMESURE *et al.* 1996; COMPS *et al.*, 2001) offer a quite clear picture of major temperature shifts, spreading of ice cover and, as a consequence, the change of spatial distribution of species. Ice ages acted in selecting variability and in changing distribution of genotypes across the continent.

In general for European beech (*Fagus sylvatica* L.) the coexistence of two major refugia, in Southern Italy and in the Balkan peninsula is accepted (DEMESURE *et al.* 1996). The colonisation of Central and North Europe after the ice retreat began from these two areas. Pollen data suggest that for Southern Italy at least two main refugia can be individuated at Laghi di Monticchio (WATTS *et al.* 1996) and Canolo Nuovo (near Reggio Calabria) (DRESCHER-SCHNEIDER 1985). Nevertheless other data suggest that also in the central part of the peninsula other refugia were present (MAGRI 1998).

The northward migration processes were in some cases limited by the presence of the main orographic barriers, in particular the Alps. Analysis of molecular marker variation has the potential to contribute to study the postglacial colonisation history in situations where the history of population migration has left behind genetic traces in present day populations (FERRIS *et al.* 1998).

The present geographic distribution of organelle markers tends to give a clearer picture of past migration history than nuclear markers, because effective population size and gene flow following range establishment are reduced in haploid, uniparentally inherited organelle markers relative to diploid, biparentally inherited markers. On the other hand random markers like the

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RAPD ones are high sensibility tools able to detect within population levels of genetic variability to be evaluated from a point of view of Vavilov's theory of centre of origin (VAVILOV 1951).

In this paper Italian populations of beech were analysed, with the main objectives (i) to test the hypothesis that populations close to the putative refugia (southern Italian populations) are characterised by higher diversity; (ii) to detect any possible trend of variation of haplotypes diversity related to the recolonisation process of the Italian peninsula. For these specific objectives, both chloroplast PCR/RFLP and RAPD markers were used.

MATERIALS AND METHODS

Thirty Italian beech populations (Table 1) were analysed with both RAPD (30 individuals for each population) techniques and PCR-RFLP (5 individuals for population) of chloroplast DNA. Extraction of DNA was performed on dormant buds (100 mg starting material) with the NucleoSpin Plant Kit (Macherey Nagel, Germany).

Amplifications were performed in 20 ml for PCR as reported by PAFFETTI *et al.* (2001) and in 25 ml for RAPD as reported by PAFFETTI *et al.* (1996).

The primer pairs for the intergenic spacers between the *trn*D-*trn*T (DT) (DEMESURE *et al.* 1995) and *orf*184-*pet*A (OA) (GRIVET *et al.*, 2001) cpDNA regions were used. Random primers 1253 (5'-GTT TCC GCC C -3'), 1247 (5'- AAG AGC CCG T -3') and RF2 (5'- CGG CCC CTG T -3') were used. Amplification products were checked by gel electrophoresis on 1 % (cpDNA) or 2 % (RAPD) (w/v) agarose gel stained with ethidium bromide. The gels were photographed with an UVP scanner (Photo-Capt, Vilbert Lourmat, France). The DT and OA amplification products were digested with *Hae*III and *Hinf*I (Takara, Japan), respectively. The mixtures were analysed by gel electrophoresis on 8 % (w/v) non-denaturating polyacrylamide gels. The digestion profiles of the two

Table 1. Italian beech (*Fagus sylvatica* L.) populations sampled along the natural range following a north-south gradient.

	Populations	Long.	Lat.	Altitude (m)
1	Foresta Umbra (Vieste, FG)	16.10	41.53	700
2	Monte Mauro (Castelmauro, CB)	14.43	41.50	1000
3	La Montagnola (Civitanova del Sannio, CB)	14.26	41.38	1050
4	Campitello Matese (S. Massimo, CB)	14.24	41.31	1500
5	Monte Taburno (Montesarchio, BN)	14.38	41.04	1000–1100
6	Laghi di Monticchino (Rionero Vulture, PZ)	15.39	40.56	650
7	Santuario M. Vergine (Mercogliano, AV)	14.44	40.55	1150–1350
8	Monte Raiamagra (Bagnoli Irpino, AV)	15.04	40.50	1200
9	Passo Crocelle (S. Fele, PZ)	15.35	40.48	1100
10	Monte Faito (Vico Equense, NA)	14.25	40.40	1100
11	Bosco Ponte Pasquino (Laurenzana, PZ)	16.02	40.24	1000
12	Località Il Parco (Viggiano, PZ)	15.57	40.23	1200
13	Faggeto (Moliterno, PZ)	15.54	40.10	1200
14	Bosco Canicello (Lauria, PZ)	15.55	40.05	850
15	Cugno Cumone (Terranova Pollino, PZ)	16.16	39.59	1300–2000
16	Acqualonga (Fuscaldo, CS)	16.07	39.25	1000
17	Fago del Soldato (Camigliatello Silano, CS)	16.27	39.20	1500
18	Bosco del Gariglione (Taverna, CZ)	16.42	39.17	1600
19	Sila di Tasso (Spezzano Sila, CS)	16.24	39.17	1400
20	Monte Pecoraro (Serra San Bruno, CZ)	16.20	38.35	1200
21	Monte Zomaro (Cittanova, RC)	16.05	38.21	950
22	Monte Basilicò (Podargoni, RC)	15.53	38.09	1400
23	Bosco Savoca (Isnello, PA)	14.05	37.56	1800
24	Bosco Savoca (Isnello, PA)	14.05	37.56	1400
25	Faggeta Pirao (Randazzo, CT)	14.50	37.53	2000
26	Bella Fontana (Caronia, ME)	14.20	37.53	1300
27	Portella Creta (Caronia, ME)	14.26	37.50	1500
28	Cozzo del Filatore (Petralia Sottana, PA)	14.10	37.48	1600
29	Cozzo del Filatore (Petralia Sottana, PA)	14.10	37.48	1300
30	Bosco Chiuso (Maletto, CT)	14.50	37.47	1800

fragments were compared simultaneously with the digestion fragments of control DNA of known haplotypes (kindly provided by Rémy Petit, INRA, Pierroton, France and by Thomas Geburek, FBVA, Vienna, Austria).

Parameters of genetic diversity (h_s and h_t) and differentiation (G_{st}) in the chloroplast genome were estimated following the methods described by Pons and Petit (1995, 1996). The contribution of a given population to total diversity (C_t) was calculated as reported by Petit *et al.* (1997). Parsimonious analysis was performed by Dollo maximum parsimony (Farris 1977) using DNAPARS program of the PHYLIP version 3.5c package (Felsenstein 1993). Analysis of molecular variance (AMOVA, Excoffier (1992), in the Arlequin 2000 software package) was performed on a presence/absence vector of RAPD markers to test the level of within and among population genetic variability. The genetic relationships among populations were analysed by neighbor-joining (Saitou & Nei 1987) using the NEIGHBOR-JOINING program of the PHYLIP version 3.5c package.

Table 2. Description of the 5 haplotypes identified with the combination of PCR/RFLP DT and OA fragments.

Haplotypes	Polymorphic fragments	
	DT	OA
3	1	4
5	2	5
8	2	4
9	4	5
11	5	5

RESULTS

The cpDNA haplotypes diversity in natural Italian populations of beech (*Fagus sylvatica* L.) was analysed using PCR-RFLP technique. Populations have been completely studied with the DT and OA primers/restriction enzymes *Hae*III and *Hinf*I combination,

Figure 1. Geographical distribution of chloroplast haplotypes in *Fagus sylvatica* L. southern Italian populations.

respectively, and a total of 5 different haplotypes (Hap) have been identified (Table 2). The parsimonious analysis reported in Figure 1 clearly individuate Hap 5 as the ancestor (haplotypes differ from each other by one mutation event). The geographic distribution of the cpDNA haplotypes of the studied populations is reported in Figure 2. The most frequent haplotypes are: Hap 5 and Hap 9. In particular Hap 5 is homogeneously distributed in Southern Italian populations, and Hap 9 is located in the very close Calabrian populations (Monte Pecoraro, Bosco del Gariglione, Sila di Tasso, Fago del Soldato, and Acqualonga). Some rare haplotypes are present at level of single individual/s (ind/s) in some population/s (pop/s) (Hap 3: 2 inds in Monte Faito and Monte Zomaro pops; Hap 8: 1 ind in Laghi di Monticchio and Monte Mauro pops; and Hap 11: 1 ind in Fago del Soldato pop) or at level of pop (Hap 3: Bosco Canicello pop).

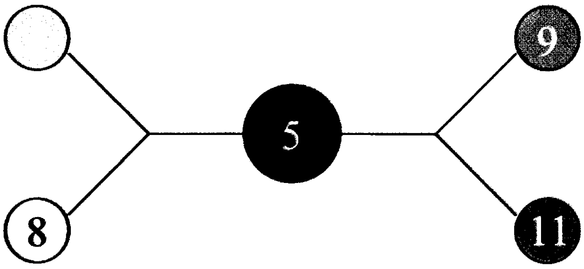

Figure 2. Maximum parsimony tree of chloroplast haplotypes.

Most of the cpDNA differentiation is distributed among populations (G_{st} = 77.25%). The Bosco Canicello, (C_t = 0.104), Monte Pecoraro (C_t = 0.087), Bosco del Gariglione (C_t = 0.087), Sila di Tasso (C_t = 0.087), Acqualonga (C_t = 0.087), Fago del Soldato (C_t = 0.066), and Monte Zomaro (C_t = 0.022) populations have contributed much more to the total diversity. In some populations intrapopulation polymorphism is also found (pops: Laghi di Monticchio, Fago del Soldato, Monte Mauro, Monte Faito, and Monte Zomaro).

RAPD amplification (with 3 primers) has generated 93 reproducible markers used in molecular variance analysis. AMOVA procedure (Table 3) has generated percentage values of differentiation within populations (67 %) higher than among population (33 %). Wright's fixation index F_{ST} is however consistent (0.33). An Euclidean distance matrix (Table 4) has been calculated to estimate values of interpopulation (for each pair of population) and intrapopulation genetic variability. Data show a high dispersion of values from the average (8.78 for interpopulation and 17.1 for intrapopulation).

A neighbour-joining tree (Figure 3) has been constructed using an among population Euclidean distance matrix. The dendrogram shows 4 clusters of populations but a geographical coherency of groups is not observable. An interesting exception is the genetic similarity of populations at different altitude at the same locations in Sicily.

DISCUSSION

Southern Italian populations of beech clearly show a high amount of genetic variability at the chloroplast level (5 different haplotypes) indicating substantial differentiation among populations (G_{ST} = 77.25%). Variability is also evident within populations with some chloroplast haplotypes that are simultaneously present in the same population. This is remarkable for a non-recombinant, uniparentally inherited, slow mutation rate genomic components such us the chloroplast.

Observing the distribution of cpDNA variants, only haplotype 5 is quite homogeneously present in the studied area. This haplotype was probably the most common before ice-ages representing, as shown in the maximum parsimony tree, the ancestral haplotype (Figure 2) that survived in many little refugia at high altitude during the warm interglacials or in quite wide basal formations (maybe at sea level) during the ice ages. This latter consideration suggest also that the whole southern part of Italy acted as a refugium; considering that, at these latitudes, ice ages caused probably "only" altitude shift of species distribution. Rare Haplotypes are randomly distributed and could represent the sign of a geographic isolation, but it's hard to think an exclusive post glacial differentiation of

Table 3. Molecular variance analysis.

Source of variation	d.f.	Sum of squares	F-test	Variance components	Percentage of variation
Among populations	29	3094.4	12.72	4.23	33
Wihin populations	667	5759.3		8.63	67
Total	696	8853.7		12.86	100

populations especially at chloroplast level, so probably beech stands were already differentiated before ice ages. Haplotype 9, common and exclusive of the central zone of Calabria, represent an interesting disjunction in the distribution of haplotype 5 and can testimony past isolation and differentiation. The presence, southward of Calabrian disjunction, of Hap. 5 in Sicily strengthen the hypothesis of its pre ice-ages existence in the island. Haplotype 3 is interesting because, although rare, is well distributed and has great influence on total interpopulation diversity.

High values of genetic distance at intrapopulation level can be observed also with RAPD markers. However the aspect that must be stressed is the high value of interpopulation distance (considering also the nature of these markers that are especially designed to detect intrapopulation variability) that is substantially higher than those generally reported in literature for European stands. Populations of beech are quite differentiated (high F_{ST} value) in Southern Italy. This fact can be ascribed to the orography of the territory which have restricted the gene flow to few and/or small areas. This is corroborated by the geographical incoherence of NJ tree clusters. Moreover the genetic similarity between low and high altitude in Sicily suggest that after the last ice age refugial populations at low altitude spread uphill to highest altitude.

In conclusion cpDNA and RAPD data are coherent in indicating high values of genetic variability at intrapopulation level and substantial diversity among stands not detected in others regions of Western Europe (Cuguen *et al.* 1987; Comps *et al.* 1990; Konnert 1995; Leonardi & Menozzi 1994, 1996; Larsen 1996; Belletti & Lanteri 1996; Sander *et al.*, 2001). This

Table 4. Euclidean matrix of among (below diagonal) and within (diagonal) population genetic distance (see numbering of populations in Table 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	**16**																													
2	6	**21**																												
3	4	3	**17**																											
4	3	3	1	**18**																										
5	10	10	10	8	**15**																									
6	11	10	10	9	7	**18**																								
7	5	3	2	2	8	8	**20**																							
8	5	7	5	4	7	8	5	**20**																						
9	5	5	3	4	11	12	2	5	**20**																					
10	3	3	2	2	11	10	3	6	4	**22**																				
11	11	13	11	9	4	10	9	6	10	13	**12**																			
12	6	9	7	8	17	17	7	9	7	5	19	**18**																		
13	5	5	3	4	11	10	4	5	3	3	13	6	**22**																	
14	11	10	12	10	4	10	10	7	10	13	5	18	12	**13**																
15	4	8	5	6	12	12	4	7	5	5	12	6	5	13	**17**															
16	5	3	4	5	13	12	5	10	7	3	17	8	6	16	7	**21**														
17	4	5	5	4	8	9	4	4	4	4	9	9	5	11	7	7	**22**													
18	6	8	6	5	6	9	5	3	5	7	5	12	7	7	9	11	6	**16**												
19	8	5	4	6	14	13	5	11	6	4	18	7	5	18	7	4	8	11	**20**											
20	9	7	8	7	9	9	7	6	8	9	12	16	7	8	10	11	7	8	13	**13**										
21	9	11	10	8	8	6	8	6	11	10	8	15	10	11	11	11	8	7	14	10	**14**									
22	13	15	13	11	6	8	11	6	12	15	4	20	14	5	14	19	11	7	21	12	8	**11**								
23	5	8	6	7	13	14	6	7	4	5	13	4	5	12	6	8	7	8	7	11	14	15	**21**							
24	7	7	6	7	16	14	6	8	3	6	15	6	6	14	8	8	7	9	8	12	15	17	4	**20**						
25	5	4	3	3	13	13	4	8	5	3	16	6	5	15	6	4	7	9	3	11	13	18	7	6	**18**					
26	8	6	6	6	10	11	4	7	4	7	10	10	6	11	7	9	6	9	8	8	11	13	8	7	8	**19**				
27	5	3	3	3	9	11	4	7	5	4	11	9	5	11	8	5	5	7	5	7	11	14	8	8	5	7	**18**			
28	12	12	11	10	12	11	10	9	11	11	14	18	10	13	11	13	11	11	14	6	11	15	14	13	13	12	12	**8**		
29	12	14	10	10	15	12	10	8	9	12	15	15	9	16	11	14	12	11	15	8	13	16	13	11	12	12	13	4	**7**	
30	11	13	12	10	4	7	10	7	11	14	3	19	13	4	13	17	11	7	19	11	8	2	14	16	16	12	13	14	16	**16**

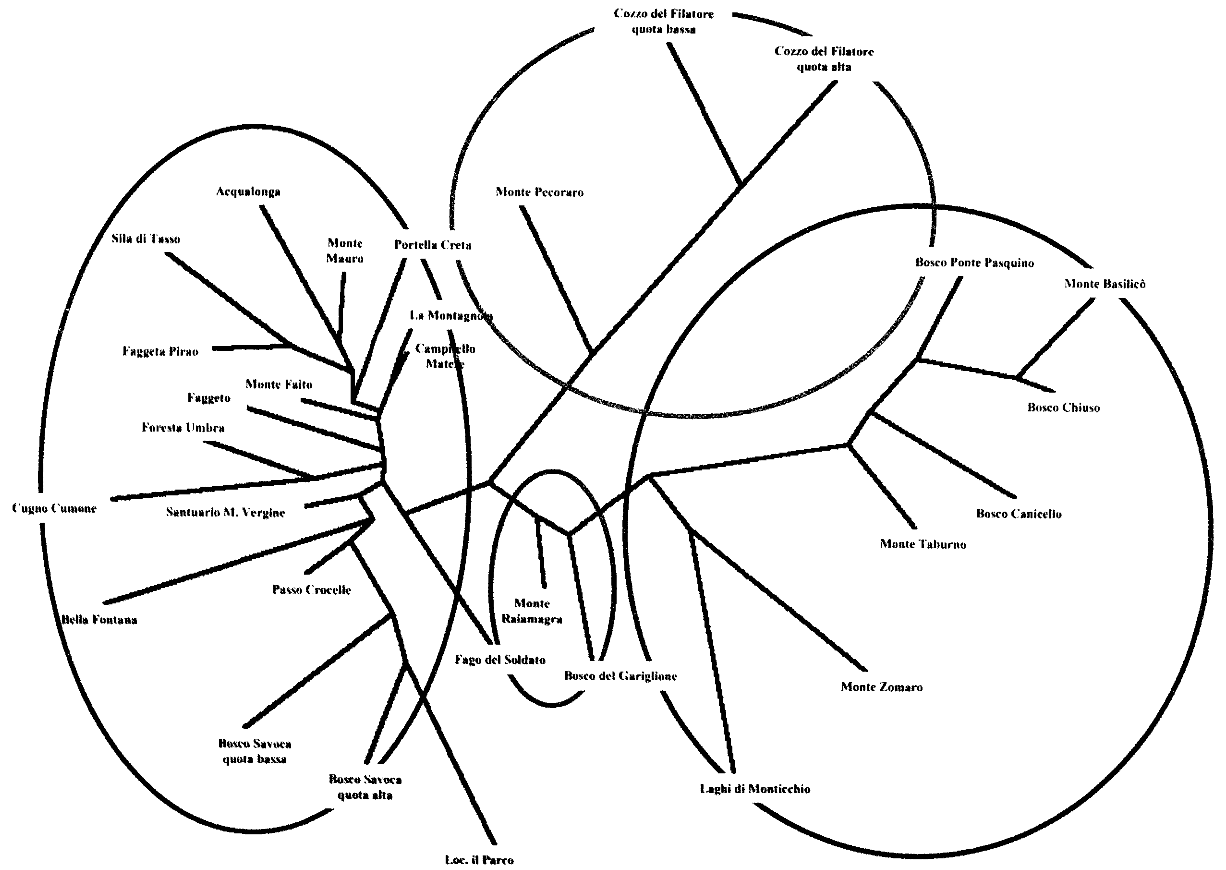

Figure 3. Neighbour-joining dendrogram of populations.

confirms a special role during ice-ages for the southern part of Italy like a reservoir of genetic richness. However, geographical distribution of haplotypes and not localised centre of variability indicate that paleoclimatic events (namely glaciations, but also interglacial period), and the orography of the territory may have determined the actual beech distribution in Southern Italy. Therefore the actual level of diversity reflects completely or in part the genetic variability present before glaciations.

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