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INHERITANCE OF RAPD AND I-SSR MARKERS AND POPULATION PARAMETERS ESTIMATION IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.)

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ABSTRACT

The Mendelian inheritance of RAPD and I-SSR markers has been assessed using a progeny from a controlled cross in European beech (*Fagus sylvatica*). Out of a total of 165 amplification products 30 Mendelian markers with dominant mode of gene action were found. A subset of 11 markers has been used to estimate population parameters in a sample of 46 trees from a natural stand in the Northern Apennines (Italy). As expected we observed higher expected heterozygosity than reported in the literature for allozyme studies of this species. The same subset of markers was used for assessing paternity of 81 seedlings belonging to 9 open-pollinated sibships of 9 individuals each. In spite of the low power of the analysis (only 2 unambiguous paternity assignments out of 46 potential fathers) its results seem in agreement with the lack of spatial clustering already known for the species.

Key words: *Fagus sylvatica*, PCR marker, inheritance, heterozygosity, paternity analysis

INTRODUCTION

Beech (*Fagus sylvatica* L.) is one of the most important European forest trees in a vast area ranging from Scandinavia to southern Italy and from Spain and southwestern France to Ukraine (TEISSIER DU CROS 1981). Allozymes have been used to study the genetic structure of beech populations (THIÉBAUT *et al.* 1982; CUGUEN *et al.* 1988; COMPS *et al.* 1990; MERZEAU *et al.* 1994; LEONARDI & MENOZZI 1995). Their characteristics (limited sampling of the genome and relatively few loci) restrict the goals of investigations carried out using this kind of genetic marker (BERGMANN 1991).

Detailed genetic profiles (obtainable thanks to the availability of a large number of DNA markers) can considerably improve our understanding of the structure and organization of genetic variability of forest tree populations and are fundamental prerequisite for QTL analysis. RAPD markers (WILLIAMS *et al.* 1990) have been widely used in population genetics studies thanks to many advantageous features: relatively simple laboratory protocols with no *a priori* specific sequence information; random, multilocus sampling of the genome; low cost-effectiveness ratio; availability of a theoretically unlimited numbers of markers. On the other hand, the dominant mode of inheritance of RAPD markers can limit their application to population genetic studies (BRADSHAW *et al.* 1994).

Recently, a new class of PCR-based, microsatellite-anchored markers has been introduced (I-SSR, Inter-Simple Sequence Repeats – ZIETKIEWICZ *et al.* 1994). These markers are amplified from highly polymorphic regions (TAUTZ & SCHLOTTERER 1994; WU & TANKSLEY 1993; MORGANTE & OLIVIERI 1993; SMITH & DEVEY 1994). In this technique a single primer targeting microsatellite sequences is used to amplify genomic sequences flanked by two inversely oriented microsatellite elements. I-SSR markers share most of the advantageous features of RAPD markers with the addition of a potentially co-dominant pattern of inheritance (ZIETCKIEWICZ *et al.* 1994) and have been considered a promising source of a large number of reliable, highly-polymorphic markers (SALIMATH *et al.* 1995).

In conifers, the analysis of (haploid) megagametophytes allows the assessment of the genetic control of DNA markers (TULSIERAM *et al.* 1992; NELSON *et al.* 1993; BINELLI & BUCCI 1994; GÖÇMEN *et al.* 1996). In non-conifer tree species, segregation analysis of progenies from controlled crosses is necessary for assessing the genetic control of the markers to be used in population genetic studies (ROY *et al.* 1992; GRATTAPAGLIA & SEDEROFF 1994; LU *et al.* 1995).

PCR-based markers have been used in population studies of many tree species (CHALMERS *et al.* 1992; BUCCI & MENOZZI 1995), but to our knowledge no reports of their use in European beech (*Fagus sylvatica* L.) exists in the literature with the exception of the description of mitochondrial and chloroplastic DNA

marker polymorphism (DEMESURE *et al.* 1995) and its recent phylogenetic development (DEMESURE *et al.* 1996). A RFLP study of chloroplastic DNA has been carried out in Beech (VORNAM & HERZOG 1996).

In this work we explore the inheritance mode of RAPD and I-SSR markers and identify a number of DNA Mendelian markers in a progeny from a controlled cross. Population parameters were estimated and a limited paternity analysis was attempted in a natural stand.

MATERIAL AND METHODS

Plant material

Plant material (leaves and cotyledons) for DNA extraction was obtained from trees in a natural stand on the Northern Apennines (Lat. 44°.24', Long. 10°.09', Alt. 1700m):

a) 22 full-sib seedlings from a controlled cross of two trees (CERONI *et al.* 1996) used for testing the genetic inheritance of markers. The 2 trees belong to a different area of the stand from which we obtained the rest of the material;

b) 46 adult beech trees for population parameter estimation and paternity analysis from the same stand located in a roughly circular patch of 100 m of radius. We sampled all the plants producing flowers and fruits within the patch;

c) 81 half-sib seedlings from open-pollinated seeds collected on 9 of the 46 trees for paternity analysis. The 9 trees were selected among plants that showed the largest number of loci with "00" genotype (see paternity analysis).

DNA isolation and amplification

DNA extraction from cotyledons and leaves was performed according to MILLIGAN (1992), with slight modifications. Approximately 40 mg of frozen (–80 °C) tissue was ground in liquid nitrogen and homogenized in 400 µl of extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA, 0.5 M NaCl, 0.2% ß-mercaptoethanol, 1% PVP). After the addition of 100 µl of SDS 2%, the homogenate was incubated at 65 °C for 20 min, mixed with 170 µl of 5 M K-acetate (pH 6.5), kept on ice for 5 min and centrifuged at 13,000 × g for 40 min. The aqueous phase was recovered and DNA was precipitated with the addition of 1/10 volume of 3 M Na-acetate and an equal volume of isopropanol for 2 hours at –20 °C. The DNA pellet was washed with 70% ethanol twice, dried, and re-suspended in 50 µl of TE buffer (10 mM Tris-HCl pH 8, 0.1 mM EDTA). DNA concentration was measured with a fluorometer (CESARONE *et al.* 1979).

DNA amplifications were performed in a PTC-100/96 thermal cycler (MJ Research). RAPD reactions were carried out as described by BINELLI & BUCCI (1994). DNA amplification with I-SSR primers was performed using the following conditions: PCR Buffer (Boehringer Mannheim) 1X, 1.5 mM $MgCl_2$, 1µM primer, 0.2 mM dNTPs, 0.75 U Taq Polymerase (Boehringer Mannheim), 5 ng genomic DNA in a final volume of 25 µl. Temperature profile was: preheating 5 min at 94 °C; 40 cycles of 30 sec at 94 °C, 45 sec at 52 °C, 2 min at 72 °C; final extension 8 min at 72 °C.

The amplification products were separated by agarose gel (2%) electrophoresis stained with ethidium bromide and photographed using a Polaroid camera. The reproducibility of the amplification patterns was verified amplifying samples at least twice and comparing the amplification profiles.

Eighty random decamers (Operon Technologies, Alameda, CA) and 41 microsatellite-anchored primers (NAPS Unit, University of British Columbia) were tested for polymorphysm in parental genotypes. Ten RAPD primers and 9 I-SSR primers were chosen on the basis of the number, sharpness and reproducibility of polymorphic bands for further genetic analysis. Sequences of the primers used in the screening of the progeny from the controlled cross are reported in Table 2.

Genetic inheritance of polymorphic bands

The genetic control of the amplification fragments generated by the 19 primers chosen was tested on 22 full sibs from a controlled pollination experiment.

All unambiguous, polymorphic amplification products were scored as dominant markers ('1': presence; '0': absence). For a Mendelian trait from a *Aa* × *aa* cross, a 1:1 segregation ratio is expected, while from a *Aa* × *Aa* cross a 3:1 segregation ratio is expected. Goodness-of-fit of the observed segregation ratios to the above expectations was tested by χ^2 test ($\alpha < 0.05$). Markers showing significant deviations were discarded from further analysis. Independence of loci was tested by Fisher's exact test ($\alpha < 0.01$).

Population parameter estimation

A subset of 7 primers selected from the original 19 for the clarity of their results (see Table 4 for a list) was used for investigating the genetic structure of the 46 adult trees and half-sib seedlings. Expected heterozygosity (H_{exp}), and its sampling variances were calculated as described by LYNCH & MILLIGAN (1994). Fragments with $Nq^2<3$ were excluded from the analysis. Devia-

tions from Hardy-Weinberg expectations (HWE) were verified by χ^2 test ($\alpha = 0.05$) by comparing the observed parental marker frequencies (46 adult-stage individuals) against the observed offspring marker frequencies (half-sib families), under the assumption of random mating (LYNCH & MILLIGAN 1994).

Paternity analysis

Paternity analysis was performed following the fractional method developed by DEVLIN *et al.* (1988). "Transition" (or Mendelian) probabilities are reported in Table 1. When a parental individual shows a '1' phenotype (band observed), under HWE, the probability of being heterozygous $P(h)$ can be computed as: $2q(1-q)/(2q(1-q) + (1-q)^2)$, and the probability of being homozygous $P(o)$ as $1-P(h)$, where q is the frequency of the '0' allele estimated as the square root of the frequency of the '0' (no band observed) phenotype in the population. It should be noted that the only case in which a putative male parent could be excluded from paternity as genetically incompatible is the third case reported in Table 1 (LEWIS & SNOW 1992). The same subset of markers used for population parameter estimation and an appropriate choice of open pollinated sibships that would maximize the number of mothers with the most informative phenotype (0) was selected for this analysis.

The probability of being the "true" father is estimated as the product, over all loci, of each "transition" probability calculated for each locus, i.e. assuming independence of loci (markers unlinked). To avoid bias due to undetermined phenotypes (missing data), only cases with a minimum of 7 loci were considered.

The distribution of pollen migration distances was computed locating seedlings at their mothers' sites (seeds were collected before dissemination).

RESULTS

A different number of primers (and markers) were used for different parts of the analysis. Nineteen primers (that eventually yielded 30 mendelian markers) were used in the assessment of the genetic control of amplification products obtained from 22 full sibs from a controlled pollination experiment. Seven primers (that we knew could reveal 11 mendelian markers) were used for population parameters estimation and paternity analysis on 46 trees and 81 open pollinated seedlings belonging to 9 sibships.

Genetic inheritance of polymorphic bands

A total of 165 amplification products were obtained. Fifty (30.3 %) were polymorphic in the 2 parents, with an average of 2.63± 0.41 polymorphic bands per primer (Table 2). Thirteen bands showing faint amplification products were not considered for further analysis. Segregation analysis of the remaining 37 brightest bands is reported in the Table 3. We considered Mendelian markers the 30 fragments (18 RAPD and 12 I-SSR) that did not show significant deviations from the expected segregation ratios (χ^2 test, $p > 0.05$). Thirteen out of 18 RAPD markers fit a 1:1 segregation ratio and the remaining 5 a 3:1 ratio. Eleven out of 12 I-SSR markers fit a 1:1 segregation ratio while the remaining one fits a 3:1 ratio. The average number of Mendelian

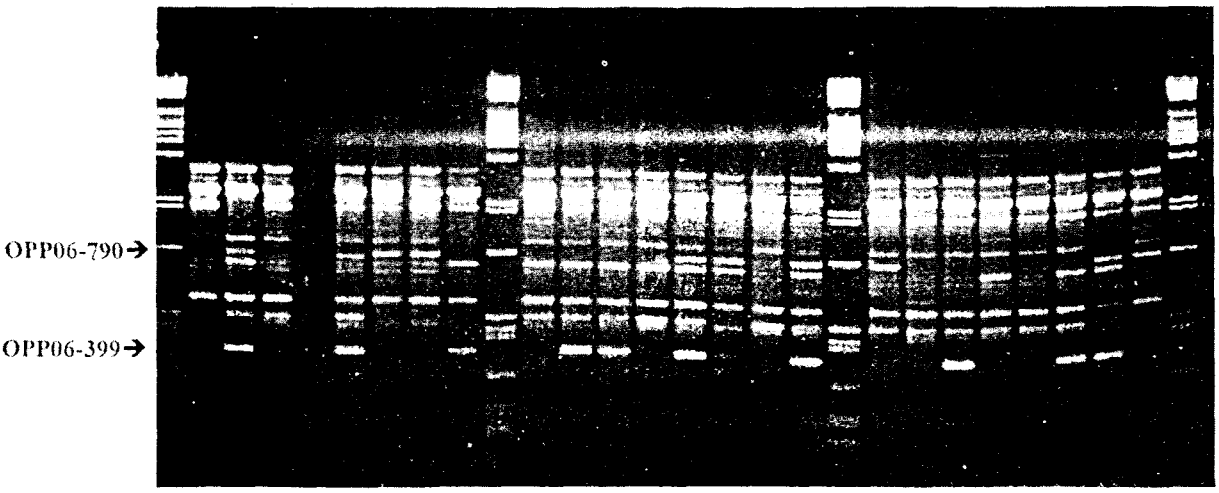

Figure 1 Amplification patterns obtained using primer OPP06 for the two parents of and 22 seedlings of the controlled cross. Lanes 1, 10, 19 and 28 hold the molecular marker λ-DNA digested with *PstI*, lanes 2 and 3 the 2 parents, lanes 4–9, 11–18, 20–27 the 22 full-sibs of the cross. The size of the fragments considered in the segregation analysis are indicated on the right. Only those of 790 bp and 399 bp that fit a 1:1 segregation ratio can be considered genetic Mendelian markers.

Table 1 "Transition" probabilities estimate paternity probabilities for each combination of mother, offspring and putative father phenotypes. Example for a dominant 1 locus 2 alleles system

Mother		Offspring		Father		"Transition" probability
Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	
0	00	**0**	00	**0**	00	1
0	00	**0**	00	**1**	11 01	*P(o) 0+* *P(h) 0.5*
0	00	**1**	01	**0**	00	*0*
0	00	**1**	01	**1**	01 11	*P(h) 0.5+* *P(o) 1*
1	01	**0**	00	**0**	00	*1*
1	01	**0**	00	**1**	01	*P(h) 0.25 P(h)*
1	01 11	**1**	01	**0**	00	*P(h) 0.5+* *P(o) 1*
1	01	**1**	01	**1**	01	*P(h) 0.5 P(h) +* *P(h) 0.5 P(o) +* *P(h) 0.25 P(h) +* *P(h) 0.5 P(o) +*
	11		11		11	*P(o) 0.5 P(h) +* *P(o) 0 P(o) +* *P(o) 0.5 P(h) +* *P(o) 1 P(o)*

Note: Paternity is a product of the probabilities estimated for all loci tested. In the above example for phenotype **1** (presence of a band) heterozygous (01) and homozygous (11) genotypes were considered.

The contribution to paternity of the locus of the above example is the sum of the probabilities of all crosses of the different genotypes compatible with the given parental phenotypes. The proportion of offspring of given phenotype from a cross is indicated by the numeric coefficient. In the more complex cases probabilities of crosses that produce 0 offspring are omitted.

segregating loci per primer was 1.33 and 1.80 for I-SSR and RAPD, respectively. Amplification patterns obtained using primer OPP06 are shown in Fig 1 as an example of our bands' quality.

A total of 435 (n (n–1)/2) two-locus combinations were tested for independent segregation. Only 4 pairwise combinations (M814–0760/OK14–0749; M814–0720 /OK14–0728; M814–1076/OW12–0484; OW12–0500/OW12–0484) showed some evidence of non-independent assortment of alleles in the gametes (Fisher's exact test; $p < 0.01$).

Population parameters

To assess the degree of polymorphism detectable in our natural population, 46 adult trees and 81 half-sib seedlings were screened using a subset of 6 RAPD and 1 I-SSR primers for a total of 11 markers. All selected markers showed no significant deviation from Hardy-Weinberg expectations as tested by the differences in frequencies between parental and offspring population (χ^2-test, $p > 0.05$).

The estimate of mean expected heterozygosity (10 loci) in the parental population was 0.300 ± 0.062, while in the half-sib population (11 loci) was 0.291 ± 0.049 (Table 4). Estimation of sampling variance components of average expected heterozygosity revealed that about 4 per cent of the total variance is due to the sampling of a finite number of individuals per locus, while about 96 per cent is due to variation in expected heterozygosity among loci (LYNCH & MILLIGAN 1994).

Paternity analysis

For 2 out of 81 seedlings, no compatible father was found within the 46 potential adults sampled from the population (Fig 2). This is evidence of an external

Table 2 Results of the amplification using 10 RAPD primers and 9 I-SSR primers for the two parents and the 22 full-sib seedlings from the controlled crosses

Primer	Sequence (5'–3')	Amplification product	Polymorphic bands	Mendelian loci
M809	$(AG)_8G$	8	1	1
M814	$(CT)_8A$	13	3	3
M822	$(TC)_8A$	8	2	2
M842	$(GA)_8YG$	10	1	0
M843	$(CT)_8RA$	15	7	1
M845	$(CT)_8RG$	14	5	3
M849	$(GT)_8YA$	16	5	1
M850	$(GT)_8YC$	6	2	0
M854	$(TC)_8RG$	3	1	1
OPW05	GGCGGATAAG	6	2	2
OPW07	CTGGACGTCA	5	2	2
OPW09	GTGACCGAGT	6	1	1
OPW11	CTGATGCGTG	10	4	2
OPW12	TGGGCAGAAG	7	4	3
OPW19	CAAAGCGCTC	7	1	1
OPP06	GTGGGCTGAC	9	4	2
OPP07	GTCCATGCCA	5	1	1
OPP08	ACATCGCCCA	8	1	1
OPK14	CCCGCTACAC	9	3	3
Total		165	50	30
Mean		8.684	2.631	1.579
S.E.		0.823	0.406	0.220

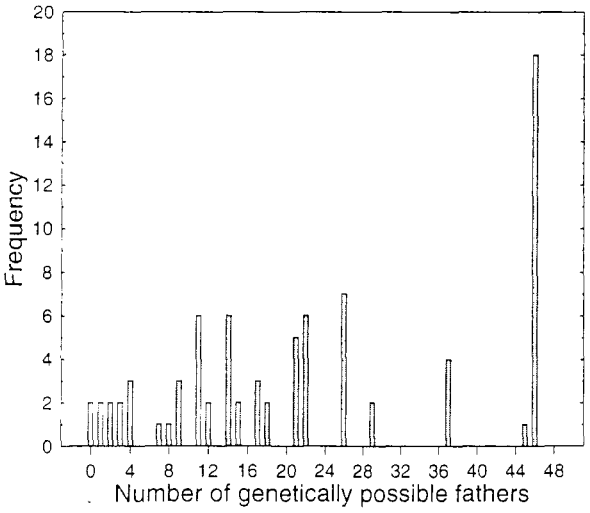

Figure 2 Sum of the number of genetically plausible male parents for 81 seedlings

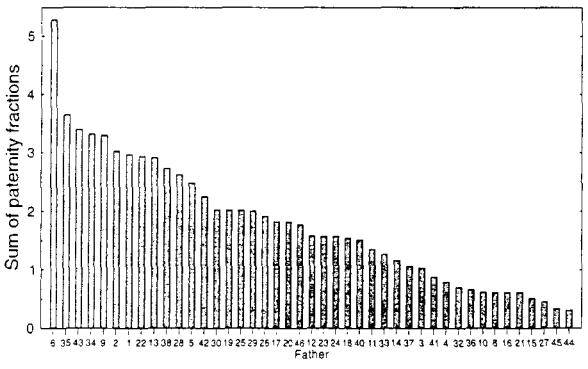

Figure 3 Sum of paternity fractions over all seedlings calculated for each of the 46 possible fathers

source of pollen for these two seedlings. For other two seedlings only one possible father was found at a distance of 29.3 meters and 29.7 meters, respectively. For all other seedlings, more than one genetically compatible father was found, and we assigned the computed paternity fraction of paternity to each potential pollen source.

Mean distance between offspring and compatible fathers (31.7m) is significantly greater than mean distance from incompatible fathers (28.6m) (Wilkoxon test: $z = -3.82163$; $p = 0.0001$).

For each potential father a sum of fractional paternities over all seedlings has been calculated. Results are shown in Fig 3. We considered the sum of paternity fractions for each adult an estimate of relative male reproductive success (SNOW & LEWIS 1993). Plant #6 has the maximum reproductive success with 2 complete paternities and fractional contributions adding up to

Table 3 Results of segregation analysis of polymorphic amplification products in the 22 full sib seedlings from the controleld cross obtained using 10 different RAPD primers and 9 different I-SSR primers

Marker	Parental phenotype		Observed			χ^2
			Presence	Absence	Total	
Inter-Simple Sequence Repeats (I-SSR markers)						
M809–0323	0	1	14	5	19	3.368^{ns}
M822–0830	1	0	8	13	21	0.762^{ns}
M822–0850	0	1	10	11	21	0.000^{ns}
M842–0286	1	1	9	11	20	8.066***
M843–0618	1	0	10	11	21	0.000^{ns}
M849–0709	0	1	13	9	22	0.409^{ns}
M850–0286	0	1	20	2	22	13.136***
M854–0636	0	1	16	6	22	3.682^{ns}
M845–0470	1	0	13	7	20	1.250^{ns}
M845–0747	1	0	11	9	20	0.050^{ns}
M845–0796	0	1	14	6	20	2.450^{ns}
M814–0760	1	0	9	10	19	0.000^{ns}
M814–0720	0	1	6	13	19	1.895^{ns}
M814–1076	1	1	13	7	20	0.600^{ns}
Random Amplified Polymorphic DNAs (RAPD markers)						
OW05–0509	1	0	10	12	22	0.045^{ns}
OW05–0383	1	0	8	14	22	1.136^{ns}
OW07–0988	1	0	10	12	22	0.045^{ns}
OW07–0643	0	1	13	9	22	0.409^{ns}
OW09–0533	1	0	7	14	21	1.714^{ns}
OW11–1414	1	0	18	3	21	9.333***
OW11–0691	1	1	14	7	21	0.390^{ns}
OW11–0586	1	1	18	3	21	0.778^{ns}
OW11–0377	1	1	10	11	21	7.000***
OW12–0720	0	1	12	10	22	0.045^{ns}
OW12–0535	1	1	8	14	22	15.515***
OW12–0500	1	0	10	12	22	0.045^{ns}
OW12–0484	1	1	16	6	22	0.000^{ns}
OW19–1732	1	0	13	9	22	0.409^{ns}
OP06–1153	1	0	21	0	21	19.048***
OP06–0790	0	1	8	13	21	0.762^{ns}
OP06–0739	1	1	11	10	21	4.587*
OP06–0399	0	1	9	12	21	0.190^{ns}
OP07–0887	1	1	13	6	19	0.158^{ns}
OP08–1230	1	1	18	4	22	0.242^{ns}
OK14–1442	1	0	8	10	18	0.056^{ns}
OK14–0749	1	0	8	10	18	0.056^{ns}
OK14–0728	0	1	6	12	18	1.389^{ns}

Note: For each amplification product we report: primer and molecular weight, parents' phenotype, presence and absence of a band in the F1 progeny, fit (χ^2-test) to the expected 1:1 or 3:1 segregation ratios depending on parental phenotype (01 and 11 respectively). (*) = $p < 0.05$; (**) = $p < 0.01$; (***) = $p < 0.001$.

more than 5 paternities. No correlation between plant size, measured by diameter at breast height, and this estimate of relative male reproductive success has been found.

DISCUSSION

The genetic make-up of *Fagus sylvatica* has so far been investigated using allozyme markers (CUGUEN *et al.*

Table 4 Analysis of RAPD and I-SSR markers in the adult trees (parental population) and in the open pollinated seedlings (offspring population)

Marker	Estimation of heterozygosity												H-W deviation
	Parental population						Offspring population						
	Pres	Abs	Tot	p*	q*	$H_{(exp)}$	Pres	Abs	Tot	p*	q*	$H_{(exp)}$	χ^2
OW05–0509	14	29	43	0.178	0.822	0.296	11	68	79	0.072	0.928	0.135	0.292^{ns}
OW05–0383	18	25	43	0.236	0.764	0.365	38	41	79	0.279	0.721	0.405	0.015^{ns}
OW12–0720	0	41	41	0.000	1.000	0.000	12	64	76	0.082	0.918	0.152	0.187^{ns}
OW12–0500	25	17	42	0.361	0.639	0.468	34	42	76	0.256	0.744	0.384	0.089^{ns}
OW12–0484	22	20	42	0.308	0.692	0.432	47	28	75	0.387	0.613	0.479	0.045^{ns}
OW07–0988	27	17	44	0.376	0.624	0.476	28	48	76	0.205	0.795	0.328	0.260^{ns}
OP06–0790	27	9	36	0.495	0.505	0.510	59	20	79	0.494	0.506	0.505	0.000^{ns}
OP06–0399	2	35	37	0.027	0.973	0.054	0	80	80	0.000	1.000	0.000	0.055^{ns}
OP07–0887	16	24	40	0.224	0.776	0.352	21	55	76	0.149	0.851	0.255	0.077^{ns}
OW09–0533	2	42	44	0.023	0.977	0.045	12	67	79	0.079	0.921	0.146	0.089^{ns}
M854–0636	29	2	31	–	–	–	71	6	77	0.715	0.285	0.413	–
Mean						0.300						0.291	
s.e.						0.062						0.049	
Intralocus H Var.						0.00015						0.00011	
(%)						0.04						0.04	
Interlocus H Var.						0.00367						0.00234	
(%)						0.96						0.96	

Note: For each marker we report: number of individuals analysed (Tot), number of individuals that have (Pres) or do not have (Abs) a band, frequencies of allele '1' (p*) and '0' (q*) calculated and standard error (s.e.) and intra- and inter-locus sampling variances. The sampling variance is the variance due to the sampling of a finite number of individuals at each locus (intralocus H Var.) and to the variance in heterozygosity among loci (interlocus H Var.). Deviation from Hardy-Weinberg equilibrium was tested for the 10 markers with $Nq^2 > 3$ following LYNCH & MILLIGAN (1994)

1988; COMPS *et al.* 1991; MÜLLER -STARCK & STARKE 1993; LEONARDI & MENOZZI 1995; PAULE 1995). In this study, we assessed the genetic control of 30 DNA markers in European beech, to our knowledge the largest number of markers described for this species. Marker segregation in the F_1 progeny from a controlled cross was mostly consistent with the inheritance patterns expected for Mendelian traits.

On average we found 1.8 Mendelian traits per RAPD primer, consistent with to what reported in the literature for other forest trees. In conifers using haploid material (megagametophytes) 1.7 Mendelian traits per RAPD primer were found for *Picea glauca* (TULSIERAM *et al.* 1992); 1.19 for *Taxus brevifolia* (GÖÇMEN 1996); 3.18 for *Picea abies* (BUCCI & MENOZZI 1993). In *Betula alleghaniensis* using three parental pairs and more than 15 full-sibs for each controlled cross ROY *et al.* (1992) found 9 Mendelian RAPD markers using 2 primers (4.5 per primer). In *Pinus sylvestris* LU *et al.* (1995), screening 6 RAPD primer in 4 controlled reciprocal crosses, found 19 Mendelian traits (3.17 per primer), 8 segregating with a 3:1 ratio and 11 with a 1:1 ratio.

As for I-SSR markers, no co-dominant traits were observed. This might be due to different reasons: the limited sample size of our F_1 progeny; the low separation of bands obtainable using agarose gel detection; the anchoring in 3' of I-SSR that limits the length polymorphism detectable within the di-tri or tetranucleotide regions (ZIETCKIEWICZ *et al.* 1994).

We found a mean expected heterozygosity (H_{exp} = 0.30) in line with the values found in other reports for this species. Using 9 allozymic loci on the same sample, LEONARDI & MENOZZI (1995) reported a value of 0.203; MÜLLER -STARCK (1989), using 14 allozymic markers in populations of various locality in Germany, found a mean expected heterozygosity of 0.277. COMPS *et al.* (1990) report values between 0.257 and 0.317 in 140 beech stands located in central and Mediterranean Europe using 6 enzyme loci; VYŠNÝ *et al.* (1995) found values between 0.171 and 0.247 in two sets of populations from western Ukraine using 13 and 12 allozyme loci; BELLETTI & LANTERI (in press) report an average value of 0.232 of 11 native populations from Northern

Italy at 10 allozymic loci. The slightly higher values obtained by I-SSR and RAPD markers are not surprising given the well known differences in the power of detecting genetic variability between allozymes and DNA markers (MÜLLER -STARCK & ZIEHE 1991).

The power of our paternity analysis was expected to be limited by the characteristics of RAPD markers (only 2 dominant alleles assumed per locus) and by the relatively low number of loci analyzed (LEWIS & SNOW 1992; MILLIGAN & MCMURRY 1993). Only 2.5% of paternities were unambiguously attributed. Nonetheless some information on pollen migration patterns can be inferred from the present investigation. Our results seem to suggest that the nearest plant is not the most likely source of pollen. This is in agreement with the low level of spatial genetic clustering found in beech (LEONARDI & MENOZZI 1996; MERZEAU *et al.* 1994). The sampling design seems to be more important than population phenomena in determining our results. The estimates of male reproductive success should be considered for comparison only. Plant #6, the only tree with un-shared paternities, has no particular morphological or genetic characteristic. Its location near the centre of the sampled area is probably the best explanation for its apparent higher reproductive success.

Paternity analysis can be notably increased using a greater number of loci, and choosing markers with higher recessive allele frequencies (SNOW & LEWIS 1993) and also working with information about *a priori* probabilities of paternity, such as timing of flowering or pollen-distribution curves (ADAMS *et al.* 1992).

PCR based markers are already a powerful tool for population genetic investigations. The development of codominant markers will increase the amount of information they can supply and make easier assessing the genetic control of their trasmission, a crucial step before their use in genetic studies.

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