Genetic Diversity and Temporal Genetic Structure in European Beech (*Fagus sylvatica* L.).

By K.-S. WANG

Institut für Forstgenetik und Forstpflanzenzüchtung der Universität Göttingen, Büsgenweg 2, D-37077 Göttingen, Germany.
Current address: Program in Genetics and Genomic Biology, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada

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Abstract

The genetic diversity and temporal genetic structure of four isolated European beech (*Fagus sylvatica* L.) stands from Germany were studied by using nine allozyme loci (GOT-B, IDH-A, LAP-A, MDH-B, MDH-C, MNR-A, 6-PGDH-A, PGI-B and PGM-A). The allelic multiplicity was higher in seed samples than adult trees indicating gene flow from neighbor stands. Genetic diversity ranged from 1.405 to 1.536 and average observed heterozygosity ranged from 0.239 to 0.325 for the four stands. The genetic differentiation in allelic frequencies between the seed lots produced in two different years was low (d_0 were 4.2% and 5.4%, F_{ST} were 0.9% and 1.2% for two stands $10A_{21}$ and $10A_{22}$). Genetic differentiation in allelic frequencies between seed trees and seed generations were rather low (d_0 ranged from 4.1% to 6.2%, F_{ST} ranged from -0.9% to 0.4% for two stands $10A_{21}$ and $10A_{22}$). Some aspects related to seed sampling were discussed.

Key words: Fagus sylvatica L., allozyme, genetic diversity, gene flow, temporal genetic structure.

Introduction

Genetic variation is an important attribute of forest tree populations enabling them to survive spatial and temporal variations in environmental conditions. The genetic variation and its structure within and between populations are also important in conservation and management of genetic resources and in applications in breeding and silviculture (BROWN, 1978; HATTEMER, 1987, 1988, 1990; ZIEHE et al., 1989; FINKELDEY, 1993; GREGORIUS, 1994). Genetic structure can also be an indicator of adaptation and adaptational potentials (HATTEMER and ZIEHE, 1997; ZIEHE et al., 1999).

Restricted gene flow, disruptive selection, genetic drift and historical events are responsible for population genetic differentiation in space and in time (LEVIN and KERSTER, 1974; ENDLER, 1977). The extent and pattern of genetic diversity in forest trees are strongly influenced by their mating systems and the movement of genes (gene flow) between dispersed populations of the same species (DAY, 1993).

European beech (*Fagus sylvatica* L.) is a widespread, monoecious and wind-pollinated tree species. It belongs to the major forest tree species and is of importance in ecology and economy. The genetic variation of beech populations has been investigated intensively (e.g. THIÉBAUT et al., 1982; MÜLLER-STARCK and STARKE, 1993; LEONARDI and MENOZZI, 1995; PAULE et al., 1995; HATTEMER and ZIEHE, 1996; KONNERT et al., 2000). Like many other tree species (HAMRICK et al., 1992), beech reveals a high level of genetic variation at allozyme gene loci (MÜLLER-STARCK and ZIEHE, 1991). Since its pollen can be dispersed over wide distances, genetic change can theoretically occur among very distant populations. Generally, a great intrapopulational but relatively small interpopulation variation is found (e.g. MÜLLER-STARCK and ZIEHE, 1991; TUROK, 1996; PAULE et al., 1995; LEONARDI and MENOZZI, 1995; HATTEMER and ZIEHE, 1996; ZIEHE et al., 1998; KONNERT et al., 2000). The little differentiation of populations suggests that gene flow between populations is extensive. The comparisons of genetic structures of reproducing forest stands and the produced offspring made for beech (*Fagus sylvatica* L.) proved changes of genetic structures during reproduction (e.g. MÜLLER-STARCK and ZIEHE, 1991; STARKE and MÜLLER-STARCK, 1992; HATTEMER et al., 1993; MÜLLER-STARCK, 1996; ZIEHE et al., 1998).

Studies on temporal genetic variation of European beech were few and nearly all concentrate on comparing the genetic differentiation between the seed generation and the respective adult tree population (e.g. GREGORIUS et al., 1986; MÜLLER-STARCK and ZIEHE, 1991; STARKE and MÜLLER-STARCK, 1992; HATTEMER et al., 1993; PAULE et al., 1995; LEONARDI and MENOZZI, 1995; HATTEMER and ZIEHE, 1996; MÜLLER-STARCK, 1996; TUROK, 1996; ZIEHE et al., 1998; WERDER, 2000; DOÚNAVI, 2000; KONNERT et al., 2000; JANSSEN, 2000). The present study was made to (1) estimate the genetic diversity within stands; (2) analyze the genetic differentiation between seed generation and adult trees; (3) explore the genetic differentiation between seed samples in two different years.

Materials and Methods

Seed materials and stands information

Four relatively isolated stands were used in this study. Three stands $34B_1$, $10A_{21}$ and $10A_{22}$ are located in the forest district of Escherode, Kaufunger Wald. The other stand 100C is located in the Solling, which is in the area of a research project of the Forest Ecology Center (Forschungszentrum Waldökosysteme at the University of Göttingen). Stand $34B_1$ is mixed with spruce, other stands are pure beech stands. Seeds in two different years (1994 and 1998) were collected from the ground under each adult tree (the seeds of stand 100C were collected only in 1998). Seeds were sampled around each adult tree at a radius of 3 m within three circles, which are 40 cm in diameter and are located north, southwest and southeast of the stem at angles of 120°. All of the seeds encountered in each circle were sampled. All of the trees within the stands were tagged, and the location of each tree was mapped. The principal features

Table 1. – Description of the isolated beech stands in the forest district of Escherode and in the Solling, northern Germany.

Stand	$34B_1$	$10A_{21}$	$10A_{22}$	100C
Age (years)	150	180	180	190
Area (m^2)	50mx70m	60mx130m	50mx50m	120mx160m
Altitude (m)	530	500	500	370
No. of adult trees	24	70	13	99
Distance to nearest beech stand (m)	300	50	50	500
No. of seeds (in 1994)	52	1699	137	
No. of seeds (in 1998)	511	844	202	1954

Table 2. – Allelic multiplicity (M) in adult trees and seed samples.

Stand	Stand $34B_1$			Stand $10A_{21}$			Stand $10A_{22}$			Stand 100C	
Year	Adult	S94	S98	Adult	S94	S98	Adult	S94	S98	Adult	S98
N	24	52	511	70	1699	844	13	137	202	99	1954
Gene locus											
GOT-B	2	2	2	2	2	2	2	2	2	2	2
IDH-A	2	2	2	3	3	3	2	2	3	2	2
LAP-A	4	4	5	5	5	5	4	4	4	4	5
MDH-B	3	3	3	5	4	5	2	3	4	4	4
MDH-C	2	2	2	2	2	2	2	2	2	2	2
MNR-A	2	2	2	2	3	3	2	2	2	2	4
6-PGDH-A	2	2	2	2	3	2	2	2	2	2	2
PGI-B	1	2	2	1	3	3	1	2	2	2	2
PGM-A	2	2	2	2	2	2	2	2	2	2	3
Sum	20	21	22	24	27	27	19	21	23	22	26

N refers number of adult trees, sample size of seed in 1994 (S94) and 1998 (S98).

and the seed amounts analyzed in laboratory are summarized in *Table 1*. The seed sample of stand $34B_1$ in 1994 was small because of fewer fruits within this stand in that year. The number of sampled trees for seeds is not identically with the number of adult trees, because no seed was found under part of adult trees. For stand 100C, the number of analyzed seeds in laboratory (1954) was sampled under 30 adult trees.

Isoenzyme electrophoresis

Nine enzyme coding gene loci were utilized for multilocus genotyping (GOT-B, IDH-A, LAP-A, MDH-B, MDH-C, MNR-A, 6-PGDH-A, PGI-B and PGM-A). Genetic control and mode of inheritance of the respective enzyme systems were verified previously (MÜLLER-STARCK and STARKE, 1993). Enzymes were separated from crude homogenate by standard horizontal starch gel electrophoresis.

Statistical analysis

Intrapopulation variation was studied by means of allelic multiplicity (M), genetic diversity (v), gametic diversity (v_{gam}), observed heterozygosity (H_o), total population differentiation of gene pool (δ_T) (GREGORIUS, 1978, 1987).

Homogeneity of allele frequency distributions was tested for statistical significance by the χ^2-test. Rare alleles were pooled in order to avoid problems with low expected frequencies. Genetic distance among samples was calculated using D (NEI, 1972) and d_0 (GREGORIUS, 1974). F_{ST} is the proportional deviation of overall expected heterozygosity from expected heterozygosity in the subpopulations.

Allelic multiplicity (M), genetic diversity (v), gametic diversity (v_{gam}), observed Heterozygosity (H_o), total population differentiation of gene pool (δ_T) and genetic distance d_0 were computed using the GSED program by GILLET (version 1.1, 1998). F-statistics (WRIGHT, 1969) and genetic distance D (NEI, 1972) were calculated with the computer program of BIOSYS-2 (SWOFFORD and SELANDER, 1997). The cluster analysis was performed with NTSYS version 2.00 (ROHLF, 1997).

Results

Genetic diversity within samples

The results on allelic multiplicity for four stands are presented in *Table 2*. More alleles in seeds than in adults were found at the gene loci LAP-A and PGI-B within stand $34B_1$, at gene loci MNR-A, 6-PGDH-A and PGI-B within stand $10A_{21}$, at gene loci IDH-A, MDH-B and PGI-B within stand $10A_{22}$ and at loci LAP-A, MNR-A and PGM-A within stand 100C. These new alleles in the seed samples must have come from neighbor beech stands through gene flow. There is one rare allele at locus MDH-B in adult within stand $10A_{21}$, which was not found in seed 94 but in seed 98. The identical number of alleles does not mean identical alleles because of rare allele and new alleles (e.g. S94 and S98 within stand $10A_{21}$).

Genetic diversity (v), gametic diversity (v_{gam}) and average total population differentiation (δ_T) are presented in *Table 3*. In general, the genetic diversity (v) of single loci and gene pool of the seed generation were a little higher than for the seed trees, except for the S94 within stand $34B_1$ and S98 within stand 100C.

The observed heterozygosities are shown in *Table 4*. The heterozygosity of seed generation was higher than that of seed trees, except for stand 100C. It can be seen that observed heterozygosities differ markedly among gene loci with their genetic diversity.

Temporal genetic structure

The allelic structure of seed trees and seeds of three stands ($34B_1$, $10A_{21}$ and $10A_{22}$) are presented in *Table 5, 6* and *7*, respectively. The homogeneity test in allelic frequencies among seed trees, seeds in 1994 (S94) and seeds in 1998 (S98) was performed. Genetic distance (d_0) and F_{ST} were also calculated.

Within stand $34B_1$, the differentiation between seed trees and seeds 98 is not significant but the differentiation between seed trees and seed 94 is significant at the gene loci LAP-A,

Table 3. – Genetic diversity (v), gametic diversity (v_{gam}) and average total population differentiation (δ_T).

Stand	Stand $34B_1$			Stand $10A_{21}$			Stand $10A_{22}$			Stand 100C	
Year	Adult	S94	S98	Adult	S94	S98	Adult	S94	S98	Adult	S98
N	24	52	511	70	1699	844	13	137	202	99	1954
Gene locus											
GOT-B	1.653	1.625	1.849	1.790	1.881	1.867	1.742	1.757	1.498	1.755	1.576
IDH-A	1.800	1.550	1.847	1.737	1.563	1.768	1.649	1.558	1.660	1.582	1.351
LAP-A	2.844	3.956	2.945	3.324	3.617	2.938	2.683	3.441	2.767	3.237	3.423
MDH-B	1.341	1.102	1.388	1.455	1.460	1.701	1.080	1.060	1.094	1.556	1.409
MDH-C	1.800	0.500	1.802	1.582	1.514	1.554	1.742	1.857	1.921	1.731	1.517
MNR-A	1.180	1.233	1.210	1.059	1.159	1.100	1.080	1.015	1.093	1.095	1.120
6-PGDH-A	1.133	1.080	1.073	1.137	1.159	1.181	1.080	1.191	1.115	1.437	1.259
PGI-B	1.000	1.019	1.004	1.000	1.003	1.011	1.000	1.007	1.010	1.062	1.106
PGM-A	1.882	1.942	1.862	1.923	1.975	1.983	2.000	1.687	1.897	1.800	1.673
V	1.482	1.461	1.503	1.476	1.495	1.520	1.405	1.408	1.407	1.536	1.446
v_{gam}	51.411	57.370	61.050	55.020	62.612	66.724	33.813	38.079	33.751	70.380	40.655
δ_T	0.332	0.319	0.335	0.325	0.331	0.342	0.300	0.291	0.290	0.351	0.308

MDH-C and PGM-A (*Table 5*). The differentiation between seeds of two years is significant at more loci (GOT-B, IDH-A, LAP-A, MDH-B, MDH-C, and PGM-A).

Within stand $10A_{21}$, the differentiation between seed trees and seeds 98 and the differentiation between seed trees and seed 94 are significant at the gene locus LAP-A (*Table 6*). The differentiation between seeds of two years is again significant at more loci (IDH-A, LAP-A, MDH-B, MDH-C, MNR-A, and PGI).

Within stand $10A_{22}$, the differentiation between seed trees and seeds 98 is not significant. However, the differentiation between seed trees and seed 94 is significant at locus PGM-A (*Table 7*). Just as in the two other stands, the differentiation between seeds of two years is significant at more loci (GOT-B, LAP-A, MNR-A and PGM).

In summary, within two stands ($10A_{21}$ and $10A_{22}$), the genetic differentiation among samples was small. The gene pool distance (d_0) between samples was never larger than 0.1 and the F_{ST} were smaller. Within stand $34B_1$, the genetic differentiation between S94 and seed trees, between S94 and S98 were a little larger (d_0 are 11.4% and 12%, respectively; F_{ST} are 4% and 5.1%, respectively).

Table 4. – Observed Heterozygosity (H_o).

Stand	Stand $34B_1$			Stand $10A_{21}$			Stand $10A_{22}$			Stand 100C	
Year	Adult	S94	S98	Adult	S94	S98	Adult	S94	S98	Adult	S98
N	24	52	511	70	1699	844	13	137	202	99	1954
GOT-B	0.292	0.442	0.456	0.371	0.429	0.428	0.462	0.399	0.322	0.465	0.341
IDH-B	0.500	0.385	0.501	0.343	0.372	0.445	0.231	0.380	0.391	0.343	0.254
LAP-A	0.583	0.654	0.638	0.686	0.702	0.619	0.538	0.617	0.446	0.556	0.688
MDH-B	0.250	0.058	0.267	0.257	0.220	0.327	0.077	0.068	0.089	0.333	0.256
MDH-C	0.500	0.481	0.406	0.286	0.332	0.344	0.462	0.401	0.510	0.364	0.350
MNR-A	0.167	0.212	0.192	0.057	0.143	0.092	0.077	0.015	0.089	0.091	0.110
6-PGDH-A	0.125	0.077	0.070	0.100	0.136	0.160	0.077	0.161	0.109	0.273	0.202
PGI-B	0.000	0.019	0.004	0.000	0.003	0.011	0.000	0.007	0.010	0.061	0.097
PGM-A	0.417	0.519	0.391	0.400	0.499	0.485	0.231	0.445	0.520	0.404	0.384
Gene pool	0.315	0.316	0.325	0.278	0.315	0.323	0.239	0.275	0.276	0.321	0.298

Table 5. – Allelic frequencies and homogeneity test of stand $34B_1$.

Stand		Stand $34B_1$			Adult/ S94			Adult/S98			S94/S98		
Year		Adult	S94	S98	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}
N		24	52	511									
Gene locus	Allele												
GOT-B	2	0.271	0.260	0.357	0.02	0.011	-0.015	1.50	0.086	0.005	3.96*	0.098	0.016
	3	0.729	0.740	0.643									
IDH-A	2	0.333	0.231	0.356	1.78	0.103	0.013	0.10	0.023	-0.009	6.57*	0.125	0.029
	3	0.667	0.769	0.644									
LAP-A	1	0.000	0.000	0.005	14.8**	0.322	0.054	1.30	0.066	-0.007	49***	0.287	0.063
	2	0.375	0.260	0.311									
	3	0.438	0.231	0.462									
	4	0.063	0.221	0.060									
	5	0.125	0.288	0.162									
MDH-B	1	0.125	0.019	0.077	5.19	0.106	0.051	3.46	0.061	-0.001	9.16*	0.111	0.029
	3	0.854	0.952	0.841									
	4	0.021	0.029	0.081									
MDH-C	1	0.333	0.548	0.334	6.07*	0.215	0.07	0.02	0.001	-0.012	19***	0.214	0.087
	2	0.667	0.452	0.666									
MNR-A	2	0.917	0.894	0.904	0.19	0.022	-0.01	0.08	0.013	-0.009	0.11	0.010	-0.004
	3	0.083	0.106	0.096									
6-PGDH-A	2	0.938	0.962	0.965	0.43	0.024	-0.008	0.97	0.027	0.000	0.03	0.003	-0.005
	3	0.063	0.038	0.035									
PGI-B	2	1.000	0.990	0.998	0.46	0.01	-0.008	0.09	0.002	-0.01	2.09	0.008	0.006
	3	0.000	0.010	0.002									
PGM-A	2	0.375	0.587	0.364	5.89*	0.21	0.071	0.02	0.011	-0.013	20***	0.223	0.091
	3	0.625	0.413	0.636									
Gene pool	Mean					0.114	0.040		0.032	-0.006		0.120	0.051
	SD						0.013			0.003			0.013

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. SD is standard deviation.

Table 6. – Allelic frequencies and homogeneity test of stand $10A_{21}$.

Stand		Stand $10A_{21}$			Adult/S94			Adult/S98			S94/S98		
Year		Adult	S94	S98	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}
N		70	1699	844									
Gene locus	Allele												
GOT-B	2	0.329	0.374	0.367	1.20	0.046	0.0004	0.66	0.038	-0.001	0.24	0.075	-0.001
	3	0.671	0.626	0.633									
IDH-A	2	0.293	0.221	0.290	4.12	0.072	0.010	0.67	0.009	-0.004	34.6***	0.075	0.013
	3	0.700	0.769	0.694									
	4	0.007	0.011	0.016									
LAP-A	1	0.007	0.014	0.017	10.98*	0.137	0.012	10.56*	0.114	0.007	136***	0.14	0.019
	2	0.279	0.364	0.357									
	3	0.414	0.303	0.440									
	4	0.093	0.138	0.066									
	5	0.207	0.182	0.120									
MDH-B	1	0.086	0.100	0.085	0.48	0.024	-0.004	5.98	0.099	0.015	93***	0.091	0.019
	2	0.007	0.002	0.001									
	3	0.821	0.818	0.743									
	4	0.071	0.080	0.170									
	5	0.014	0.000	0.001									
MDH-C	1	0.243	0.217	0.232	0.53	0.026	-0.002	0.03	0.011	-0.004	1.44	0.015	0.000
	2	0.757	0.783	0.768									
MNR-A	1	0.000	0.005	0.006	4.21	0.045	0.010	1.41	0.019	-0.001	15***	0.027	0.005
	2	0.971	0.926	0.953									
	3	0.029	0.069	0.041									
6-PGDH-A	1	0.000	0.001	0.000	0.27	0.01	-0.003	0.41	0.019	-0.001	2.46	0.01	0.000
	2	0.936	0.926	0.916									
	3	0.064	0.074	0.084									
PGI-B	1	0.000	0.000	0.001	0.21	0.001	-0.003	0.75	0.005	-0.001	6.10*	0.004	0.002
	2	1.000	0.999	0.994									
	3	0.000	0.001	0.005									
PGM-A	2	0.400	0.443	0.454	1.02	0.043	0.000	1.333	0.054	0.002	0.53	0.011	0.000
	3	0.600	0.557	0.546									
Gene pool	Mean					0.045	0.004		0.041	0.003		0.042	0.009
	SD						0.003			0.003			0.004

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. SD is standard deviation.

Genetic distances between samples

The genetic distances between seed trees, seeds in 1994 and seeds in 1998 for three stands were estimated based on the basis of D (NEI, 1972) and d_0 (GREGORIUS, 1974). *Table 8* shows the matrix of genetic distances. The genetic distance (d_0) indicated that there was small genetic differentiation among samples, except for the values between S94 from stand $34B_1$ and other samples. The D is smaller than d_0.

The UPGMA dendrogram based on d_0 is presented in *Figure 1.* The sample of seeds 94 of stand $34B_1$ was somewhat different from its seed trees and other samples. This is probably due to the small sample size. Seed trees and their seeds in 1994 and in 1998 within stand $10A_{21}$ showed close similarity, they were presented in one cluster. Seed trees within stand $10A_{22}$ and their seeds showed also similar structure.

Discussion

Genetic diversity

This study shows the genetic diversity (v) ranged from 1.405 to 1.536 and the total genetic differentiation (δ_T) ranged from 0.290 to 0.351. These results are slightly higher than previous results derived from beech stands (MÜLLER-STARCK and ZIEHE, 1992; PAULE et al., 1995; TRÖBER, 1995; MÜLLER-STARCK, 1996). Interestingly, allelic multiplicity in the seed generation is sometimes higher than in seed trees, especially at gene loci of LAP-A, MDH-B, MNR-A, 6-PGDH-A and PGI-B. Those values indicate that gene flow from neighbor stands is effective and resulted in an increase of allelic variants due to external pollen in seed generation as observed by MÜLLER-STARCK (1996) within the isolated beech stand 100C. HAMRICK and GODT (1989) have pointed out that levels of genetic diversity within populations were influenced by several characteristics of the species. Seed dispersal, breeding system and geographic range all have predictive value. Under the conditions of small population size, genetic drift could lead to a rapid loss of alleles, particularly rare alleles. However, the high reproductive capability, high outcrossing rate and effective gene flow may have counteracted this effect.

The average degree of heterozygosity is one commonly used measure of genetic variation of individuals and populations and provides some information on the mating system. Heterozygosity plays an important role for genetic applications in tree breeding as well as gene conservation (ZIEHE and HATTEMER, 1998). Heterozygosity has often been seen as evidence of heterosis and numerous positive correlations between allozyme heterozygosity and fitness-related traits have been reported in the literature (see review of DAVID, 1998). Heterozygosity showed greater values in the tolerant than in the sensitive subpopulations of European beech under air-pollution stress (MÜLLER-STARCK, 1989; ZIEHE et al., 1990). MÜLLER-STARCK et al. (1992) summarized several studies in European beech and reported that average heterozygosity ranged from 0.222 to 0.312. The present study revealed that heterozygosity

varied between 0.239 and 0.325 and showed slightly higher values than previously reported in European beech (MÜLLER-STARCK and ZIEHE, 1991; PAULE et al., 1995; TRÖBER, 1995; MÜLLER-STARCK, 1996). Hence, the small stands that formed the experimental material for the present study are not at all less variable than others and are fully eligible for natural regeneration.

Temporal genetic structure

The genetic distance d_0 (4.2% and 5.4%) and F_{ST} (0.9% and 1.2%) between samples drawn from the seed crops produced in two different years (1994 and 1998) for two stands $10A_{21}$ and $10A_{22}$, respectively (without the seed samples of stand $34B_1$), are low. GREGORIUS et al. (1986) showed even lower genetic differentiation between seeds of the same stand in two different

Table 7. – Allelic frequencies and homogeneity test of stand $10A_{22}$.

Stand		Stand $10A_{22}$			Adult/S94			Adult/S98			S94/S98		
Year		Adult	S94	S98	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}	X^2	d_0	F_{ST}
N		13	137	202									
Gene locus	Allele												
GOT-B	2	0.308	0.314	0.210	0.004	0.006	-0.023	0.85	0.097	0.007	8.72**	0.104	0.025
	3	0.692	0.686	0.790									
IDH-A	2	0.269	0.234	0.270	0.17	0.036	-0.018	0.06	0.003	-0.022	1.85	0.039	0.001
	3	0.731	0.766	0.728									
	4	0.000	0.000	0.002									
LAP-A	2	0.423	0.387	0.394	3.40	0.154	-0.004	1.34	0.055	-0.024	44***	0.124	0.018
	3	0.423	0.305	0.421									
	4	0.038	0.139	0.015									
	5	0.115	0.169	0.171									
MDH-B	1	0.038	0.026	0.037	0.25	0.013	-0.019	0.20	0.007	-0.019	2.15	0.017	0.000
	2	0.000	0.004	0.002									
	3	0.962	0.971	0.955									
	4	0.000	0.000	0.005									
MDH-C	1	0.308	0.361	0.399	0.30	0.054	-0.018	0.85	0.091	-0.002	0.71	0.037	0.000
	2	0.692	0.639	0.601									
MNR-A	2	0.962	0.993	0.955	2.33	0.031	0.028	0.12	0.006	-0.02	6.67*	0.037	0.021
	3	0.038	0.007	0.045									
6-PGDH-A	2	0.962	0.912	0.946	0.77	0.049	-0.007	0.12	0.016	-0.017	3.81	0.033	0.005
	3	0.038	0.084	0.054									
PGI-B	2	1.000	0.996	0.995	2.17	0.004	-0.019	1.13	0.005	-0.018	0.12	0.001	-0.003
	3	0.000	0.004	0.005									
PGM-A	2	0.500	0.288	0.384	5.00*	0.212	0.079	1.39	0.116	0.009	6.12*	0.095	0.017
	3	0.500	0.712	0.616									
Gene pool	Mean					0.062	0.003		0.044	-0.009		0.054	0.012
	SD						0.017			0.007			0.004

* P < 0.05; ** P < 0.01; *** P < 0.001. SD is standard deviation

Table 8. – Matrix of genetic distances: Genetic distance (d_0) of GREGORIUS (1974) are shown below the diagonal and genetic distance (D) of NEI (1972) are shown above the diagonal.

Stand	Samples	Stand $34B_1$			Stand $10A_{21}$			Stand $10A_{22}$		
Year		Adult	S94	S98	Adult	S94	S98	Adult	S94	S98
N		24	52	511	70	1699	844	13	137	202
Stand $34B_1$	Adult	***	0.028	0.002	0.005	0.009	0.008	0.005	0.008	0.004
	S94	0.114	***	0.030	0.030	0.029	0.038	0.022	0.026	0.020
	S98	0.032	0.120	***	0.004	0.010	0.006	0.008	0.010	0.008
Stand $10A_{21}$	Adult	0.051	0.120	0.047	***	0.004	0.003	0.007	0.010	0.010
	S94	0.071	0.112	0.067	0.045	***	0.005	0.008	0.011	0.015
	S98	0.062	0.142	0.059	0.041	0.042	***	0.008	0.017	0.016
Stand $10A_{22}$	Adult	0.053	0.097	0.066	0.061	0.073	0.062	***	0.010	0.005
	S94	0.068	0.103	0.079	0.074	0.071	0.094	0.062	***	0.006
	S98	0.046	0.097	0.067	0.067	0.088	0.085	0.044	0.054	***

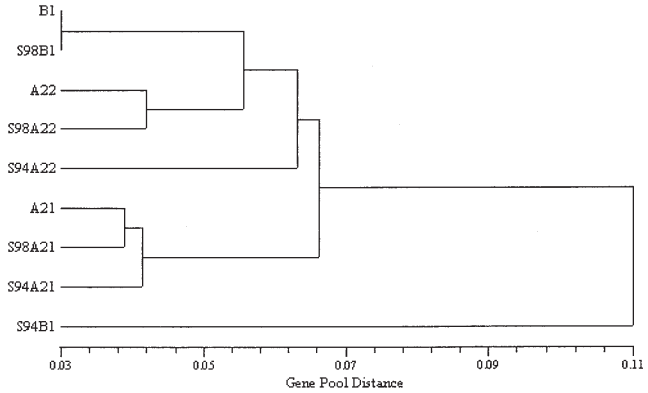

Figure 1. – UPGMA dendrogram with gene pool distance between 9 samples. Where B1, A21 and A22 denote adult trees of stands $34B_1$, $10A_{21}$ and $10A_{22}$, respectively. S94 and S98 mean seeds samples for stands $34B_1$, $10A_{21}$ and $10A_{22}$, respectively.

years (mean $d_0 = 3.1\%$ and $F_{ST} = 0.4\%$). This could result from local differences in flowering of trees in different years, as well as non-random dispersal of the seed, but the reproducing parts of the stand were genetically similar in both years (GREGORIUS et al., 1986). LEVY and NEAL (1999) reported that temporal variation in genetic structure of *Phacelia dubia* might be shaped by fluctuations in population size over time and variation in seed output among plants. The most apparent temporal changes at enzyme loci were observed in the presence and frequencies of rare alleles but no loss of rare alleles. The homogeneity of genetic structure and genetic diversity among the five age classes of Neolitsea sericea may reflect the occurrence of similar reproductive events in different years as reported by CHUNG et al. (2000).

The genetic differentiation in allelic frequencies between seed trees and seed generation were rather low (d_0 ranged from 4.1% to 6.2%, F_{ST} ranged from -0.9% to 0.4% for the two stands $10A_{21}$ and $10A_{22}$) and significant only at few loci. The genetic distances d_0 between seed trees and seed samples are slightly higher than the result reported by ZIEHE et al. (1998), which ranged from 2.8% to 5.3% and similar to the result reported by HATTEMER et al. (1993), which ranged from 2.5% to 6.5%. MÜLLER-STARCK (1996) reported slightly lower genetic distances (d_0) between seed stand and seed samples, which ranged from 1.4% to 3.5%. Genetic differentiation in time is much less marked than in space as observed by LINHART et al., 1981; GREGORIUS et al., 1986; HOSSAERT-MCKEY et al., 1996. Genetic difference between seed trees and seeds samples may be explained primarily as fertility selection and different degrees of self-fertilization (ZIEHE et al., 1998).

Seed collection

Seed collection methods have considerable impact on the genetic quality of the seed. The gene-pool of the produced seeds should be fully represented in those harvested. Gene frequencies should not be unduly distorted, and the danger of inbreeding should be at a minimum. A representative distribution of the collected lot over the stand is more easily achieved when the seeds can be collected from the ground. The exploitation of rodent hordes should be only occurring as an exception (HATTEMER and MELCHIOR, 1993). It is clear that the genetic structure of the seeds is expected to have some similarity with the parent trees by which it is produced. However, it is certainly not identical because of external pollen flow, non-random mating and selection. The seeds collected in a stand do hardly have precisely the same genotypic structure as the adult trees themselves (HATTEMER et al., 1993). The theoretical conditions for genetic equilibrium in natural population are at best approximated.

If the seeds were picked just under the sampled in the canopy trees, their allelic structures resembled those of the adult stand more closely than if they were caught in nets. This result demonstrates both the complexity and the importance of appropriate sampling of the seed population (HATTEMER, 1995). However, genetic change is implied by most operations during seed collection. The appropriate mode of sampling the seed produced by a population is rather complex. The complexity of genetic structure is partially reduced by picking the seed off the ground rather than collecting it from the trees. In beech, two out of the many conceivable approaches to seed collection were compared by ZIEHE et al. (1998).

Comparisons between stands and their progenies provided with first information about the suitability of material sampling methods. The results of this study indicated that genetic differentiation between seed trees and seed generation is not significant by most gene loci. These showed the seed samples have close genetic similarity to the parental genetic structure as observed by ZIEHE et al. (1998). It has been suggested that the seed collecting method of seeds like this study represents the seed stand well (ZIEHE et al., 1998). JANSSEN (2000) suggested that seed crop collections for conserving forest genetic resources and supplying forest enterprise with seed should be done in years of mast by considering as many as possible beech trees that are distributed over the total plot. Within four stands, multi-locus genotype exclusion analysis and parentage analysis showed that seeds, from 38.46% for seed sample of stand $34B_1$ (1994) to 78.22% for seed sample of stand $10A_{22}$ (1998) tend to fall under the seed tree and most seeds (from 65.56% to 84.79%) were dispersed within 20 m (WANG, 2001). These indicated that seed collections should be close to seed trees in order to represent the adult stands.

Sample size is an important factor in seed collection. In the present study, within stand $34B_1$, the seed sample of 1994 is small (52 seeds) and comprised seeds sampled under 6 of 24 adult trees only. The gene pool diversity (v) and total population differentiation (δ_r) are smaller than in the adult trees. The allelic differentiation between seed trees and seeds of 1994 is significant at the loci LAP-A, MDH-C and PGM-A. The gene pool distance (d_0) and F_{ST} are a little large (11.4% and 4%, respectively). For stand 100C, although the number of seeds analyzed with electrophoresis is very large (1954 seeds), these seeds were sampled only under 30 adult trees (the total number of adult trees is 99). It can be seen from *Tables 3* and *4* that the gene pool diversity, gametic diversity, total population differentiation and heterozygosity in the seeds were smaller than those in the adult stand. These results indicated that seeds should be collected under as many adult trees within a stand as ever possible. In the case of clumped spatial genetic structure these trees should be evenly distributed.

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