# ALLELIC AND GENOTYPIC VARIATION OF 13 EUROPEAN BEECH (*FAGUS SYLVATICA* L.) – POPULATIONS IN HESSE, GERMANY

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## ABSTRACT

Allelic and genotypic variation at seven different enzyme loci of European beech (*Fagus sylvatica* L.) was investigated in 13 108–160-years-old stands growing in 9 different growing areas in Hesse in central Germany.

At the loci *Aat-C*, *Acp-D*, *Acp-F*, *Dia-B*, *Idh-A*, *Lap-A* and *Per-D* 18 alleles and 34 genotypes were quantified. The rare allele B1 at locus *Dia-B* was observed in only 5 of 13 stands, the rare allele A1 at locus *Lap-A* was missing in one stand. The effective number of alleles ($N_e$) varied little from stand to stand, maximum $N_e$-values per gene locus were stand dependent. The genetic identity of the 13 stands was high, $I = 99.27$ %. Values of subpopulation differentiation ranged from $D_j = 0.025$–$0.073$. The 13 stands can be clustered to five groups according to their genetic distances $d_{o\text{-}total}$; grouping is stand dependent but independent on the growing area in which the stands occur.

Values of expected heterozygosity varied from $H_l = 5.82$ % at locus *Dia-B* to 70.92 % at locus *Lap-A*. However, heterozygosities varied little from stand to stand ($H_e = 36.98$ to 41.58 %). Of the 78 possible pairs of stands, 49 pairs (62.82 %) showed different genotype frequencies at a total of 12 genotypes. Comparing genotype frequencies in one stand with those of the 12 remaining stands showed that 10 out of 13 stands (76.92 %) differed in at least one genotype. Enlarging the number of investigated enzyme loci would probably allow to differentiate all stands.

**Key words**: beech, *Fagus sylvatica*, genetic variation, growing region, isozymes, population genetics

## INTRODUCTION

European beech (*Fagus sylvatica* L.) is the most frequent tree species in Central Europe. In Germany, it now covers an area of 1.5 million ha which corresponds to only 20 % of the original wooded area present at the beginning of the Middle Ages (SCHÜTT *et al.* 1992). Formaly, European beech comprised 80 % of the extended hardwoods in Hesse (JANßEN & WEISGERBER 1992), whereas today, not more than 30 % of this forested area is covered with beech (DERTZ 1996).

European beech's genetic variability has been investigated in France (MERZEAU *et al.* 1994; THIEBAUT *et al.* 1982; COMPS *et al.* 1987; COMPS *et al.* 1990) and Italy (LEONARDI & MENOZZI 1995; BELLETTI & LANTERI 1996) as well as in Germany (LÖCHELT & FRANKE 1995; TUROK 1994; STARKE *et al.* 1995; HATTEMER & ZIEHE 1996; KONNERT & HENKEL 1997; KONNERT *et al.* 2000) and the Balkan Peninsula (GÖMÖRY *et al.* 1999; HAZLER *et al.* 1997).

Genetic inventories within smaller regions in Germany such as Lower Saxony (MÜLLER-STARCK 1991), Nordrhein-Westfalen (TUROK 1994), Baden-Württemberg (LÖCHELT & FRANKE 1995), Rheinland-Pfalz (HATTEMER & ZIEHE 1996), Bavaria (KONNERT 1995) and Thuringia (KONNERT & HENKEL 1997), revealed extremely low genetic differences between autochthoneous beech populations. However, this does not exclude stand specific allele frequencies due to different forest management or climatic differences as shown for the black forest (LÖCHELT & FRANKE 1995) nor does it exclude stand specific genotype frequencies as described for the Vogelsberg-region in Hesse (SANDER *et al.* 2000).

The aim of this study was to analyse 13 climax beech populations in Hesse (central Germany) to see whether allele and genotype frequencies within stands might correlate with the growing areas into which Hesse has been subdivided.

## MATERIAL AND METHODS

### Stand characteristics and sampling

The state of Hesse has been subdivided into 12 growing areas (cf. Figure 2). In 9 areas 13 beech stands, aged

**Table 1. Characteristics of the 13 European beech stands investigated in Hesse, central Germany.**

| Code | Growing area | Forest district, stand | Altitude (m) | Age (1991) Year of regeneration | Number of investigated trees | Area (ha) | Parent rock |
|---|---|---|---|---|---|---|---|
| G | Nordhessisches Bergland | Kaufungen | 385–445 | 149 (1846) | 100 | 16.3 | Bundsandstein with loess loam |
| H | Nordwesthessisches Bergland | Wolfhagen, 4 C | 370–420 | 112 (1883) | 100 | 13.1 | mittlerer Buntsandstein with loess loam |
| I | Rhön | Hilders, 27 B1 | 800–850 | 143 (1852) | 100 | 14.8 | Phonlith with loess loam and pumice-stone |
| J | Spessart | Sinntal, 411 1 | 410–450 | 114 (1881) | 100 | 10.2 | mittlerer Buntsandstein with loess loam |
| K | Taunus | Chausseehaus, 159 1 | 420–470 | 143 (1852) | 100 | 18.9 | quarzite with loess loam |
| L | Westerwald | Weilburg, 863 | 310–370 | 149 (1846) | 100 | 17.2 | basalt with loess loam |
| M | Nördliches hessisches Schiefergebirge | Frankenberg, 257 | 390–440 | 160 (1835) | 100 | 15.1 | Zechstein with loess loam |
| N | Vogelsberg | Schotten, 277 | 280–310 | 135 (1860) | 100 | 14.2 | basalt with loess loam |
| O | Vogelsberg | Schotten, 154A | 600–660 | 131(1964) | 100 | 21.6 | basalt |
| P | Vogelsberg | Schotten, 387 | 360–400 | 108 (1887) | 100 | 11.0 | basalt with loess loam |
| Q | Wetterau and Gießener Becken | Nidda, 605 I | 210–260 | 120 (1875) | 100 | 11.2 | loess |
| R | Wetterau and Gießener Becken | Nidda, 415 A | 150–180 | 114 (1881) | 100 | 9.1 | loess |
| S | Wetterau and Gießener Becken | Nidda, 23 B | 172–203 | 160 (1835) | 100 | 5.9 | basalt with loess loam |

Buntsandstein (new red sand stone), Zechstein (upper permian)

between 108 and 160 years, were investigated (Table 1). Within each stand a grid of 22.5 × 22.5 m was measured, and between December and February twigs were taken from the crown of the trees nearest to grid points, harvesting a total of 100 trees per stand (JANßEN & WEISGERBER 1992). The twigs were put in water to which a little charcoal was added and stored in the dark at + 4 °C until enzyme extraction.

## Enzyme extraction

Several buds were separated per twig of an individual tree and 150 mg of yellow-green twig-leaves were homogenized and centrifuged according to SANDER *et al.* 2000. The supernatant was transferred in an Eppendorf-safe-lock-tube, shock frozen in liquid nitrogen, and stored at –80 °C until electrophoresis.

## Electrophoresis

Aspartate aminotransferase (EC 2.6.1.1), acid phosphatase (EC 3.1.3.2), diaphorase (EC 1.6.4.3), isocitrate dehydrogenase (EC 1.1.1.42), leucine aminopeptidase (EC 3.4.11.1) and peroxidase (EC 1.11.1.7) were separated by polyacrylamide gradient gel (PAGG) electrophoresis. Linear PAGG ranged from 4 to 22 % T (T = gram acrylamide + gram Bismethyleneacrylamide; acrylamide: Bis = 24 : 1) (ROTHE 1994). Per gel

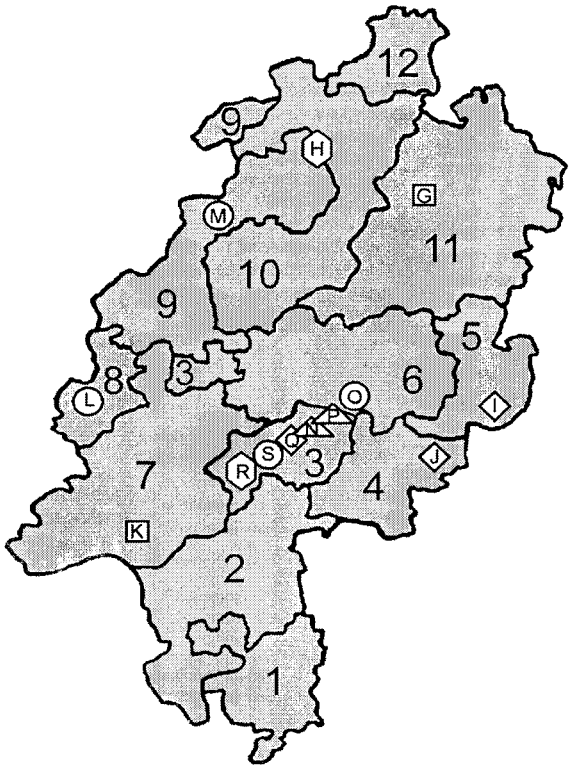


**Figure 1.** Outline of Hesse, location of investigated stands (capital letters) and growing areas (numbers) as given in the legend to Figure 1. Uninvestigated areas: 1 – Odenwald, 2 – Hessian Rhein-Main-plain, and 12 – Weserbergland.

(172 × 82 × 2 mm) 25 samples could be run. As gel buffer served a solution of 450 mM Trishydroxymethylaminomethane, 400 mM boric acid and 125 mM EDTA-$Na_2$, pH 8.4. As electrode buffer a 1 in 10 diluted gel buffer was used. After pre-electrophoresis of 15 min, 8 ml of crude enzyme extract were applied per gel trough and run at 300 V for 5 ½ h at 4 °C. Following electrophoretic separation, gels were stained for enzymes as described by Soltis *et al.* (1983) (aspartate aminotransferase), HARRIS & HOPKINSON (1976) (diaphorase, isocitrate dehydrogenase) and ROTHE (1994) (leucine aminopeptidase, acid phosphatase, peroxidase).

### Genetic interpretation of enzyme patterns and genetic measures

The investigated enzyme systems were also submitted to starch gel electrophoresis (cf. SANDER *et al.* 2000) and enzyme patterns hereafter interpreted according to MERZEAU *et al.* 1989; MÜLLER-STARCK & STARKE 1993; and MÜLLER-STARK personal communication. The genetic interpretation of enzyme patterns after polyacrylamid gradient gel electrophoresis were done in comparison to the patterns obtained by starch gel electrophoresis.

The following population genetics parameter were estimated using the formula given in the literature (cf. summation in ROTHE 1994): effective number of alleles, $N_e$ (CROW & KIMURA 1970), genetic identity $I$ (NEI 1972), genetic distances, $d_o$ (GREGORIUS 1974; BERGMANN 1974), subpopulation differentiation, $D_j$ (GREGORIUS & ROBERDS 1986), Nei's coefficients of genetic diversity, $H_T$, $H_S$, $D_{ST}$, $G_{ST}$ (NEI 1973), Hardy-Weinberg distributions of genotypes (BARTELS 1971) using the degrees of freedom as given by FERGUSON (1980), expected heterozygosities, $H_I$ (FALKENHAGEN 1985) and $H_e$ (NEI & ROYCHOUDHURY 1974) and significance of genotype frequencies (von MELTZER & ROTHE 2000). Cluster analyses were performed by use of the unweighted pair-group arithmetic mean (UPGAM) method (FERGUSON 1980).

## RESULTS

### Average alleles per locus

At the 7 polymorphic loci *Aat-C*, *Acp-D*, *Acp-F*, *Dia-B*, *Idh-A*, *Lap-A* and *Per-D*, a total of 18 alleles (cf. Tables I to VII) was found which corresponds to an average of 2.57 alleles per locus.

### Stand dependent allele frequencies

Aspartate aminotransferase (*Aat*) is coded by three gene loci in beech (SANDER *et al.* 2000).

Two alleles were attributed to locus *Aat-C* (Table I, Appendix). MÜLLER-STARCK and STARKE (1993) described two further alleles at this locus but these could not be detected in our material. Our alleles $C_1$ and $C_2$ correspond to alleles $C_2$ and $C_3$ described by MÜLLER-STARCK & STARKE (1993). Near our allele $C_1$ a weak secondary isozyme was seen on the zymograms. Its existence was deduced from a comparison of starch-gel and PAGG-gel patterns. Private alleles, alleles which occur exclusively in a single population, were not observed at this locus.

Acid phosphatase (*Acp*) is encoded by at least eight loci in beech (SANDER *et al.* 2000) of which loci *Acp-D* (2 alleles) and *Acp-F* (2 alleles) were quantified (Table II and III, Appendix). Private alleles could not be observed.

Diaphorase (*Dia*) is coded by two gene loci in beech (SANDER *et al.* 2000) of which locus *Dia-B* with three alleles was quantified (Table IV, Appendix). The rare Allele $B_1$ ($p = 0.01$) occurred exclusively in stands I, K, N, O, and Q.

Isocitrat dehydrogenase (*Idh*) is coded by one locus *Idh-A*, comprising two alleles (SANDER *et al.* 2000).

They correspond to alleles *Idh-A*$_2$ *and Idh-A*$_3$ described in the literature; allele $A_1$ and $A_4$ described by MÜLLER-STARCK and STARKE (1993) could not observed. Close to the band which corresponds to allele $A_1$ a small band was observed which is a secondary isozyme. Private alleles were not found at this locus (Table V, Appendix).

Isozymes of leucine aminopeptidase (*Lap*) were attributed to two loci (SANDER *et al.* 2000) of which *Lap-A* was quantified (Table VI, Appendix). Four alleles were attributed to locus *Lap-A*. A fifth allele (MÜLLER-STARCK & STARKE 1993) could not be observed. Allele $A_1$ was missing in stand R.

According to the zymograms obtained in PAGG-gel electrophoresis three loci code for peroxidase isozymes (*Per*) (SANDER *et al.* 2000). One locus, *Per-D*, with three alleles was quantified (Table VII, Appendix). In starch-gel electrophoresis only two *Per*-loci were observed; here, locus *Per-B* comprising two alleles corresponded to locus *Per-D* (with three alleles) in PAGG-gel electrophoresis. Private alleles could not be observed in Hessian beech.

### Allelic variation between stands

**Rare alleles:** Rare alleles ($p < 5$ %) were observed at locus *Lap-A* (Table VI, Appendix) where the mean frequency of allele A1 over all stands was 3.92 %. The lowest frequency of allele A1 at locus *Lap-A* was observed at the two lowest elevated stands (R (150–180 m above sea level) $p = 0$ %; S (172–203 m) $p = 1$ %). However, the highest frequency, $p = 7$ % was not found at the highest elevated stands I (800–850 m) and O (600–660 m) but at stands M (390–440 m) and N (280–310 m). Rare allele frequencies were also observed at locus *Dia-B* (Table IV, Appendix), where allele B1 showed an average frequency over all stands of $p = 0.39$ %. This allele was not found at 8 (stands G, H, J, L, M, P, R, S) of the 13 investigated stands and at the remaining 5 stands its frequency was 1 %. In the stands N to S at mount Vogelsberg (growing regions Wetterau and Gießener Becken and region Vogelsberg) allele B1 at locus *Dia-B* was observed in 3 stands (N, O, Q) out of 6 stands while at the other 7 stands it was seen in only 2 (I, K) stands.

**Effective number of alleles, $N_e$:** The effective number of alleles varied little from stand to stand (Table 2). Over all scored loci the mean value over the 13 stands was $N_e = 1.7884$; the maximum *$N_e$-value* (1.869) was calculated for stand G, while the lowest *$N_e$-value* (1.688) was found for stand H. Minimum and maximum values at one of the investigated loci were not regularly distributed among stands [*Aat-C*: $N_e min$ = 1.6968 (stand P), $N_e max$ = 1.9231 (stand R); *Acp-D*: min = 1.3932 (stand R), max = 1.8546 (stand K); *Acp-F*: min = 1.3047 (stand K), max = 1.8036 (stand G); *Dia-B*: min = 1.0618 (stand M), max = 1.2851 (stand I); *Lap-A*: min = 2.823 (stand H), max = 3.3574 (stand G) and *Per-D*: min = 1.5052 (stand P), max = 1.7831 (stand O)].

**Table 2. Effective number of alleles ($N_e$) of the 13 beech stands in Hesse.**

| Stand | $N_e$ at locus | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|
| | *Aat–C* | *Acp–D* | *Acp–F* | *Dia–B* | *Idh–A* | *Lap–A* | *Pod–D* | |
| G | 1.9321 | 1.6507 | 1.8036 | 1.0832 | 1.7119 | 3.3574 | 1.5433 | 1.8689 |
| H | 1.7961 | 1.4706 | 1.4706 | 1.1285 | 1.6088 | 2.8230 | 1.5212 | 1.6884 |
| I | 1.8349 | 1.6756 | 1.5614 | 1.2851 | 1.5402 | 3.1852 | 1.6442 | 1.8180 |
| J | 1.8778 | 1.7705 | 1.6128 | 1.1959 | 1.6389 | 3.1878 | 1.5123 | 1.8280 |
| K | 1.8902 | 1.8546 | 1.3047 | 1.1278 | 1.7026 | 3.3428 | 1.6991 | 1.8460 |
| L | 1.8824 | 1.7476 | 1.5095 | 1.0940 | 1.5535 | 2.9886 | 1.7017 | 1.7825 |
| M | 1.8562 | 1.7001 | 1.4706 | 1.0618 | 1.5011 | 3.2420 | 1.6141 | 1.7780 |
| N | 1.7313 | 1.6000 | 1.3717 | 1.1391 | 1.5738 | 3.2438 | 1.5310 | 1.7415 |
| O | 1.8579 | 1.7241 | 1.5267 | 1.1638 | 1.7676 | 3.0687 | 1.7831 | 1.8413 |
| P | 1.6968 | 1.7928 | 1.4235 | 1.1628 | 1.4866 | 3.0547 | 1.5052 | 1.7318 |
| Q | 1.8890 | 1.4706 | 1.5675 | 1.1874 | 1.5545 | 3.1248 | 1.5294 | 1.7605 |
| R | 1.9231 | 1.3932 | 1.6000 | 1.1271 | 1.7122 | 2.8765 | 1.7194 | 1.7645 |
| S | 1.9030 | 1.7476 | 1.6201 | 1.0832 | 1.5614 | 2.9629 | 1.7176 | 1.7994 |
| Mean | 1.8516 | 1.6614 | 1.5264 | 1.1415 | 1.6087 | 3.1122 | 1.6171 | 1.7884 |
| *n* | 2 | 2 | 2 | 3 | 2 | 4 | 3 | 2.5714 |

*n:* observed number of alleles at the respective gene locus. Total effective number of alleles (geometric mean of all $N_e$ 's): $N_{e\text{-}total}$: 1.7136

**Genetic identity, I:** The average total genetic identity of the 13 beech stands in Hesse is very high (99.27 %). It varied from 98.34 % (stands H/K) to 99.93 % (stands L/S). At the 7 loci the average genetic identities between stands were: *Aat-C*: 99.59 %, *Acp-D*: 99.20, *Acp-F*: 99.38, *Dia-B*: 99.92, *Idh-A*: 99.66, *Lap-A*: 97.40, *Per-D*: 99.74.

**Genetic distances between stands, $d_o$:** The average total genetic distances $d_{o\text{-}total}$ calculated from the distances $d_o$ at the 7 enzyme loci investigated were very low ($d_o$ = 5.45 %) (Table 3). They varied from $d_{o\text{-}total}$ = 1.75 % (stands L and S) to 8.61 % (stands G and P). Minimum and maximum $d_o$-*distances* at one of the seven loci varied stand specific (Table 3).

**Subpopulation differentiation, $D_j$:** The average genetic distance $d_o$ of one stand (subpopulation) was also compared with the average $d_o$-value of the remaining complement of the 12 populations. The resulting gene pool differentiation is low ($D_j$ = 2.53–7.30 %). Where more than one stand was investigated per growing area (stands N, O and P of the Vogelsberg region, and stands Q, R and S of the region of the

**Table 3. Genetic distances ($d_o$) at seven enzyme loci of the 13 beech stands in Hesse.**

| Enzyme system | Locus | Genetic distance ($d_o$) | | |
|---|---|---|---|---|
| | | Mean (%) | min: stands (%) | max: stands (%) |
| Acid phosphatase | *Acp–D* | 6.56 | 0.32: H,Q | 19.18: K,R |
| | *Acp–F* | 5.69 | 0.00: H,M | 20.00: G,K |
| Aspartate aminotransferase | *Aat–C* | 3.98 | 0.07: K,Q | 11.76: G,P |
| Diaphorase | *Dia–B* | 2.98 | 0.00: G,S | 9.63: I,M |
| Isocitrate dehydrogenase | *Idh–A* | 4.17 | 0.01: G,R | 11.25: O,P |
| Leucine aminopeptidase | *Lap–A* | 10.12 | 1.14: I,Q | 20.19: H,N |
| Peroxidase | *Per–D* | 4.64 | 0.41: H,N | 9.60: O,Q |
| $d_o$-*total* | | 5.45 | 1.75: L,S | 8.61: G,P |

Mean(%): average genetic distance over all populations at the respective gene locus, min (%): minimum genetic distance between named populations, max (%): maximum genetic distance between named populations, $d_o$-*total*: total genetic distance over all loci investigated.

**Table 4. Subpopulation differentiation ($D_j$) of 13 beech stands in Hesse.**

| Stand | Subpopulation differentiation $D_j$ | | | | | | | Gene pool |
|---|---|---|---|---|---|---|---|---|
| | *Aat–C* | *Acp–D* | *Acp–F* | *Dia–B* | *Idh–A* | *Lap–A* | *Pod–D* | |
| G | 5.33 | 0.66 | 11.58 | 3.00 | 3.92 | 10.54 | 3.42 | 5.06 |
| H | 3.00 | 8.25 | 2.50 | 0.83 | 0.42 | 11.50 | 3.30 | 4.26 |
| I | 1.17 | 0.42 | 1.83 | 6.75 | 2.58 | 0.75 | 4.17 | 2.53 |
| J | 1.00 | 4.75 | 4.00 | 2.42 | 1.75 | 22.10 | 3.42 | 5.63 |
| K | 2.08 | 9.08 | 9.00 | 0.83 | 3.92 | 12.71 | 3.08 | 5.82 |
| L | 1.00 | 3.67 | 0.34 | 1.92 | 20.58 | 6.04 | 3.08 | 5.23 |
| M | 0 | 1.50 | 2.50 | 4.08 | 22.75 | 7.92 | 0.17 | 5.56 |
| N | 6.58 | 2.83 | 6.84 | 0.25 | 18.50 | 13.83 | 2.25 | 7.30 |
| O | 0.08 | 2.58 | 0.34 | 1.33 | 16.83 | 4.66 | 5.33 | 4.45 |
| P | 7.67 | 5.83 | 4.67 | 1.33 | 17.75 | 4.00 | 3.34 | 6.37 |
| Q | 2.08 | 8.25 | 1.84 | 2.42 | 2.58 | 1.00 | 5.58 | 3.39 |
| R | 4.25 | 11.50 | 2.92 | 0.83 | 3.92 | 7.17 | 3.17 | 4.82 |
| S | 3.17 | 3.66 | 4.00 | 3.00 | 1.50 | 5.00 | 3.33 | 3.38 |
| $C_j$ | | | | | | | | 4.91 |

Gene pool = average over all loci at one stand; $C_j$ = average gene pool value

**Table 5. Nei's coefficients of genetic diversity, total genetic diversity $H_T$, average genetic diversity within ($H_S$) and between ($D_{ST}$) stands and percent of $D_{ST}$ on $H_T$ ($G_{ST}$).**

| | *Aat–C* | *Dia–B* | *Idh–A* | *Lap–A* | *Per–D* | *Acp–D* | *Acp–F* | Mean |
|---|---|---|---|---|---|---|---|---|
| $H_T$ (%) | 46.12 | 12.67 | 37.88 | 68.56 | 38.16 | 39.98 | 34.66 | 39.72 |
| $H_S$ (%) | 45.92 | 12.19 | 37.66 | 67.78 | 37.95 | 39.40 | 34.08 | 39.28 |
| $D_{ST}$ (%) | 0.20 | 0.48 | 0.22 | 0.78 | 0.21 | 0.58 | 0.59 | 0.44 |
| $G_{ST}$ (%) | 0.43 | 3.79 | 0.58 | 1.14 | 0.55 | 1.45 | 1.70 | 1.38 |

$D_{ST} = H_T - H_S$; $G_{ST} = D_{ST} / H_T$

Wetterau and Gießener Becken) $D_j$-*values* are not region but stand dependent (Table 4).

At a single locus the lowest differentiation is found at locus *Aat-C* ($D_j$ = 0, stand M) while the highest differentiation is observed at locus *Idh-A* ($D_j$ = 22.75 %, stand M). At locus *Aat-C* stand P deviates most from the remaining 12 stands ($D_j$ = 7.67 %), at locus *Acp-D* it is stand R ($D_j$ = 11.50 %), at locus *Acp-F* stand G ($D_j$ = 11.58 %), at locus *Dia-B* stand I ($D_j$ = 6.75 %), at locus *Idh-A* stand M ($D_j$ = 22.75 %), at locus *Lap-A* stand J ($D_j$ = 22.10 %) and at locus *Per-D* stand Q ($D_j$ = 5.58 %) which deviates most from the remaining 12 stands. At each investigated locus there is one specific stand which deviates most from the compliment of the remaining stands.

**Nei's coefficients of genetic diversity $H_T$, $H_S$, $D_{ST}$ and $G_{ST}$:** The mean of the total genetic diversity, $H_T$ (NEI 1973) was calculated to be $H_T$ = 39.72 % (Table 5). Depending on the investigated gene locus, $H_T$-*values* ranged from 12.67 % (*Dia-B*) to 46.12 % (*Aat-C*). The total genetic diversity results almost entirely from the diversity within populations ($H_S$) for which a value of $H_S$ = 39.28 % was determined. The genetic diversity between stands is, therefore, extremely low ($D_{ST}$ = 0.44 %). NEI's genetic differentiation between stands, the $G_{ST}$-*value*, confirms this result ($G_{ST}$ = 1.38 %). Accordingly, the coefficient of genetic differentiation within stands is extremely high: 98.62 %. The lowest value of $G_{ST}$ was calculated for locus *Aat-C* (0.43 %, Table 5) which means that this locus contributes among the investigated ones least to the total diversity ($H_T$). The largest $G_{ST}$-value was obtained for locus *Dia-B* (3.79 %, Table 5).

**Cluster analysis:** The method of UPGMA (unweighted pair-group arithmetic average) was used to cluster the 13 stands according to their genetic distances ($d_o$) (Figure 1). They group into five classes with increasing genetic distances (1: Q, I, J; 2: S, L, M, O; 3: R, H; 4: P, N and 5: G, K) (Figure 2).

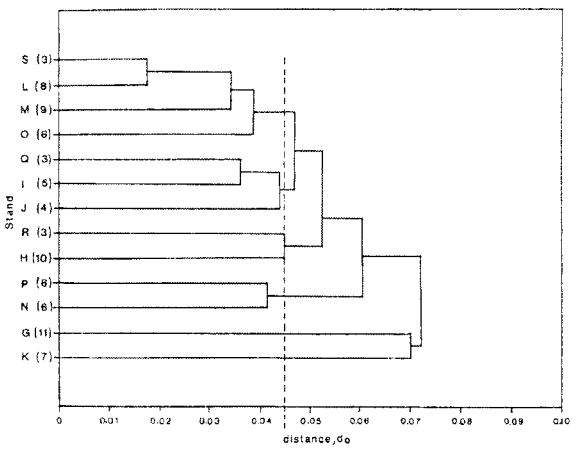


**Figure 1.** Dendrogram of genetic distances (average over 7 loci, $d_{o\text{-}total}$) of the 13 Hessian beech populations. Capital letter: stand code, in brackets: stand name, number of growing area and growing area: G (Kaufungen; 11: N-O-Hessian hills), H (Wolfhagen; 10: N-W-Hessian hills), I (Hilders; 5: Rhön), J (Sinntal; 4: Spessart), K (Chausseehaus; 7: Taunus), L (Weilburg; 8: Westerwald), M (Frankenberg; 9: N-O-Hessian Schiefergebirge), N (Schotten; 6: Vogelsberg), O (Schotten: 6), P (Schotten: 6), Q (Nidda; 3: Wetterau and Gießener Becken), R (Nidda; 3) and S (Nidda; 3). Grouping of stands: 1: S, L, M, O; 2: Q, I, J, 3: R, H, 4: P, N and 5: G, K.

## Genotypic variation between stands

**Hardy-Weinberg distributions of genotypes:** Comparing the observed genotypes at the 7 loci to those expected according to Hardy-Weinberg's theorem, no significant deviations were found when applying the $\chi^2$-test ($\alpha$ = 0.05), except for the genotypes at locus *Lap-A* (Table VI, Appendix). Here, significant deviations ($\alpha$ > 0.01) were found in stands, H, J, L, M, N, O and S.

**Pairs of stands with significantly different genotype frequencies**: At the 7 loci investigated, a total of 34 genotypes (average genotypes over all stands per polymorphic locus = 4.86) were observed (Table I-VII, Appendix). Of these genotypes 19 occurred with different frequencies in several pairs of stands (Table 6). Eighteen pairs differed in the frequency of one genotype, 20 in two genotypes, 6 in three genotypes, 2 in four genotypes, 2 in five genotypes and 1 in six genotypes (Table 6). According to these differences 49 pairs of stands out of 78 differ in frequency of at least

**Table 6. Pairs of stands with significantly ( $\alpha \leq 5\%$) different genotype frequencies.**

| | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | 7,10,11 | | | | | | | | | | | |
| I | 8 | | | | | | | | | | | |
| J | | | | | | | | | | | | |
| K | 4,5,6,17 | 3,10,11,14 | 19 | 5,6,18 | | | | | | | | |
| L | 16,17 | 12 | 8,9 | | 10,14 | | | | | | | |
| M | 5,11 | 10 | 8,9 | | 11 | 12 | | | | | | |
| N | 1,2,5,6,16 | 15 | 19 | | 10 | | | | | | | |
| O | 5,17 | 11 | | | 10,14 | | 11 | 14 | | | | |
| P | 2,5,10 | 3,12 | | | 10,14 | | 10,12 | | | | | |
| Q | | 13 | | | 3,4,10,14,18 | 12,13 | | | 13 | 3,4,12 | | |
| R | 10,11 | 12 | 3 | 3,4 | 3,4,5,10,11,14 | 3,4 | 3,10,12 | 4,14 | 3,11 | 3,4 | 12 | |
| S | 10,17 | | 8,19 | | 5,6,10 | | 10 | | | | | 3,4 |

1: *Aat-$C_1C_1$*; 2: *Aat-$C_1C_1$*
3: *Acp-$D_1D_1$*; 4: *Acp-$D_1D_2$*
5: *Acp-$F_1F_1$*; 6: *Acp-$F_1F_2$*; 7: *Acp-$F_2F_2$*
8: *Dia-$B_1B_2$*; 9: *Dia-$B_2B_3$*;
10: *Lap-$A_1A_1$*; 11: *Lap-$A_1A_3$*; 12: *Lap-$A_1A_4$*; 13: *Lap-$A_2A_3$*; 14: *Lap-$A_2A_4$*; 15: *Lap-$A_3A_4$*; 16: *Lap-$A_4A_4$*
17: *Per-$D_1D_3$*; 18: *Per-$D_2D_3$*; 19: *Per-$D_3D_3$*

one genotype (62.82 %).

Stands with significantly different genotype frequencies in relation to the remaining complement of 12 stands: At the seven loci investigated, a total of 12 genotypes differed in frequency between one stand and the remaining complement of 12 stands (Table 7). Five stands (I, J, M, P, S) differed in frequency at one genotype from the remaining stands, one stand (G) in two genotypes, three stands (H, Q, R) in 3 genotypes and one stand (K) in six genotypes. All together 10 stands out of 13 differed from the remaining 12 stands. On the other hand, stand I differed at genotype *Dia $B_2B_2$* from the rest as did stand M, but frequencies of genotype *Dia $B_2B_2$* were different in both stands (I = 77 %, rest: 89 %; M = 94 %, remainder: 87 %. In stands P and S the frequency of genotype *Lap $A_1A_2$* was zero (Table 7). Thus eight out of 13 stands (61.54 %) can be distinguished from the remaining complement of 13 stands. Three stands (L, N, O) showed no different genotype frequencies as compared to the remaining 12 stands. Enlarging the investigated number of genotypes would provide the chance to distinguish all of the 13 stands.

Average heterozygosities, $H_I$: The average expected heterozygosities at the seven loci varied considerably, namely from $H_I$ = 5.82 % at locus *Dia-B* to 70.09 % at locus *Lap-A*. At the other 5 loci values ranged from 23.36 % (*Acp-F*) to 48.24 % (*Aat-C*). Mean values over all loci, however, differed hardly from stand to stand; they ranged from 36.40 % (stand P) to 41.58 % (stand O).

## DISCUSSION

The genetic variability of the 13 investigated stands in Hesse in central Germany is similar to that of other European bech (*Fagus sylvatica* L.) populations in Germany (LÖCHELT & FRANKE 1995; TUROK 1994; STARKE *et al.* 1995; HATTEMER & ZIEHE 1996; KONNERT & HENKEL 1997; KONNERT *et al.* 2000) and other European countries (LEONARDI & MENOZZI 1995; BELLETTI & LANTERI 1996; MERZEAU *et al.* 1994; THIEBAUT *et al.* 1982; COMPS *et al.* 1987; GÖMÖRY *et al.* 1999). At the seven enzyme loci investigated, a total of 18 alleles was determined which equals 2.57 alleles per locus (A/L). The value depends on the type and number of enzymes investigated. For German beech populations A/L quotients from 2.3 (MÜLLER-STARCK

**Table 7. Significant ($\alpha \leq 5$ %) differences in genotype frequencies between one stand and the remaining complement of 12 stands.**

| Stand (m) | Genotype | | | | | | | | | | | | Σ | Combination |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Acp-* $D_1D_1$ | *Acp-* $D_1D_2$ | *Acp-* $D_2D_2$ | *Acp-* $F_1F_1$ | *Acp-* $F_1F_2$ | *Dia-* $B_2B_2$ | *Lap-* $A_1A_2$ | *Lap-* $A_2A_3$ | *Lap-* $A_2A_4$ | *Lap-* $A_3A_4$ | *Lap-* $A_4A_4$ | *Per-* $D_2D_3$ | | |
| G (415) | | | | # | | | | | | | # | | 2 | 4/11 |
| H (395) | | | # | | | | $\#_0$ | | | | | | 3 | 3/7/10 |
| I (825) | | | | | | # | | | | | | | 1 | 6 |
| J (430) | | | | | | | | | | | | # | 1 | 12 |
| K (445) | # | # | | # | # | | # | | # | | | | 6 | 1/2/4/5/7/9 |
| L (340) | | | | | | | | | | | | | – | – |
| M (415) | | | | | | # | | | | | | | 1 | 6 |
| N (295) | | | | | | | | | | | | | – | – |
| O (630) | | | | | | | | | | | | | – | – |
| P (380) | | | | | | | $\#_0$ | | | | | | 1 | 7 |
| Q (235) | # | | | | | | | # | | | | # | 3 | 1/8/12 |
| R (165) | # | # | | | | | $\#_0$ | | | | | | 3 | 1/2/7 |
| S (188) | | | | | | | $\#_0$ | | | | | | 1 | 7 |

m: altitude of stand (meter above sea level);
$\#_0$: the genotype frequency in the stand equals zero;
combination: combination of genotypes with significant unequal frequencies.

1991) to 2.85 (Sander *et al.* 2000) were calculated. Among the loci investigated only one, *Aat-B*, was found to be monomorphic, so that 7 out of 8 enzyme loci were polymorph (88 %). This value also depends on the type and number of enzymes investigated. For German beech populations the relative number of polymorphic loci was calculated to range from 79 % (LÖCHELT & FRANKE 1995) to 92 % (SANDER *et al.* 2000). Lower values were calculated for Italian beech populations (68 %, BELLETTI & LANTERI 1996). According to these data, the genetic variability of beech in Hesse seems not to be restricted.

Differentiation of stands can be evaluated in several different ways: a) by comparing genetic distances ($d_o$) taking allele frequencies as base and b) by comparing genotype frequencies. In each type of grouping either pairs of populations (stands) can be compared or, one population (stand) is compared to the

grouping either pairs of populations (stands) can be compared or, one population (stand) is compared to the remaining complement of investigated stands.

Genetic distances as averaged over all seven loci investigated, were low, $d_{o\text{-}total}$ = 5.45 %; they ranged from 1.75 to 8.61 %. Genetic distances were stand specific and where more than one stand was investigated in the same growing area (stands N, O and P at the growing area Vogelsberg, respectively stands Q, R and S at the growing region Wetterau and Gießener Becken) stands did not cluster to a group of its own. The genetic distance $d_o$ of one stand was also compared to the genetic distance of the remaining group of 12 populations (= sub-population differentiation, $D_j$). Values of $D_j$ which are smaller than the average value of the 12 $D_j$-values ($d$ = 0.0491) were observed for five stands (I, S, Q, H, O); they deviate less in sub-population differentiation. Values of $D_j$ which exceed the average $D_j$-value were observed for seven populations (G, L, M, J, K, P, N); they deviate gradually from the average. Stands N and P for which the highest gene pool values were observed belong to the same growing area which is the Vogelsberg region. Taking the subpopulation differentiation at each of the seven loci separately, the maximum $D_j$-values are found at different populations. At locus *Aat-C* the maximum value of $D_j$ (7.67 %) is found in stand P, at locus *Acp-D* it is stand R ($D_j$ = 11.50 %), at *Acp-F* it is stand G ($D_j$ = 11,58 %), at *Dia-B* it is stand I (6.75 %), at *Idh-A* it is stand M ($D_j$ = 22.75 %), at locus *Lap-A* it is stand J ($D_j$ = 22.1Õ %) and at locus *Per-D* it is stand Q ($D_j$ = 5.58 %). Stands Q and R belong to the same growing region which is Wetterau and Gießener Becken but stand differentiation is larger than region differentiation.

Usually allele frequencies rather than genotype frequencies are considered to judge the genetic relatedness of populations. On the other hand, enzyme genotypes and not alleles represent the catalytic entities which determine the metabolism of organisms. The 13 stands investigated in Hesse may be grouped to 78 pairs. Of these 49 differed in at least one genotype frequency (62.82 %). Most of the differences come from locus *Lap-A* (7 different genotypes) than range the loci *Acp-F* (3 genotypes), *Per-D* (3 genotypes), and finally *Aat-C* (2 genotypes), *Acp-D* (2 genotypes) and *Dia-B* (2 genotypes). Eighteen pairs of stands differed with respect to one genotype frequency, 20 pairs differed in two genotype frequencies six pairs in three genotypes, two pairs in four genotypes, two pairs in five genotypes and one pair in six genotypes. The two stands which differed most (6 genotypes) were stand K and R, then came the stand pairs K/Q and G/N (5 genotypes), then stand pairs G/K, K/H (4 genotypes), then stand pairs H/G, K/J, P/G, Q/P, R/M, and S/K (3 genotypes), then 20 pairs with differences in two genotype frequencies and 18 pairs with frequencies in one genotype.

Comparing the subpopulation differentiation as calculated by allele frequencies ($D_j$-values) with the differentiation that results when one compares the genotype frequencies at one population with that of the remaining complement of the populations, different results are obtained. The largest gene pool difference was observed for stand N, followed by P, K, J, M, L and G. Whereas with genotype frequencies the sequence is K (differences at 6 genotypes) (H, Q, R at equal range; 3 genotypes), G (2 genotypes) and (I, J, M, P, S, at equal range; 1 genotype). Genepools are no indicators of metabolic structures, whereas genotypes are. In other words gene pools indicate a potential to set up genotypes whereas genotypes indicate physiological situations.

The genotype differences may have two different causes. They may derive from different adaptations to climatic and edaphic conditions or they may be traced back to different forest managements. On the other hand, they may be selectively neutral. A decision can be made by investigation of the physico-chemical properties of the various isoforms. For Norway spruce such differences were observed for various isozyme forms coded by locus *Pep-C* (ROTHE & BERGMANN, 1995).

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## APPENDICES

**Table I. Observed genotypes (*Aat–C*) and number of investigated beech per stand.**

| Stand | Genotype frequency (*Aar–C*) | | | | W |
|---|---|---|---|---|---|
| | *N* | $C_1C_1$ | $C_1C_2$ | $C_2C_2$ | |
| G | 96 | 12 | 54 | 30 | ** |
| H | 92 | 10 | 41 | 41 | ** |
| I | 80 | 7 | 42 | 31 | ** |
| J | 98 | 15 | 43 | 40 | ** |
| K | 83 | 10 | 43 | 30 | ** |
| L | 100 | 12 | 51 | 37 | ** |
| M | 97 | 13 | 44 | 40 | ** |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: ** : α = 0.01 (incidental difference), – : α< 0.01 (non incidental difference).

**Table II. Observed genotypes at locus *Acp–D* and number of investigated beech per stand.**

| Stand | Genotype frequency (*Acp–D*) | | | | *W* |
|---|---|---|---|---|---|
| | *N* | $D_1D_1$ | $D_1D_2$ | $D_2D_2$ | |
| G | 94 | 55 | 27 | 12 | * |
| H | 96 | 62 | 30 | 4 | ** |
| I | 100 | 54 | 37 | 9 | ** |
| J | 100 | 49 | 38 | 13 | ** |
| K | 99 | 39 | 48 | 12 | ** |
| L | 100 | 47 | 43 | 10 | ** |
| M | 99 | 52 | 37 | 10 | ** |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: **: α= 0.01 (incidental difference), –: α < 0.01(non incidental difference).

**Table III. Observed genotypes at locus *Acp-F* and number of investigated beech per stand.**

| Stand | Genotype frequency (*Acp-F*) | | | | *W* |
|---|---|---|---|---|---|
| | *N* | $F_1F_1$ | $F_1F_2$ | $F_2F_2$ | |
| G | 100 | 44 | 45 | 11 | ** |
| H | 100 | 62 | 36 | 2 | ** |
| I | 100 | 59 | 35 | 6 | ** |
| J | 100 | 55 | 39 | 6 | ** |
| K | 100 | 76 | 21 | 3 | ** |
| L | 100 | 61 | 35 | 4 | ** |
| M | 100 | 64 | 32 | 4 | ** |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: **: α= 0.01 (incidental difference), –: α < 0.01(non incidental difference).

**Table V. Observed genotypes at locus *Idh–A* and number of investigated beech per stand.**

| Stand | Genotypes frequency (*Idh–A*) | | | | *W* |
|---|---|---|---|---|---|
| | *N* | $A_1A_1$ | $A_1A_2$ | $A_2A_2$ | |
| G | 78 | 8 | 30 | 40 | ** |
| H | 73 | 7 | 23 | 43 | ** |
| I | 97 | 6 | 32 | 59 | ** |
| J | 98 | 8 | 36 | 54 | ** |
| K | 67 | 6 | 27 | 34 | ** |
| L | 97 | 8 | 29 | 60 | ** |
| M | 85 | 5 | 26 | 54 | ** |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: ** : α = 0.05 (incidental difference), *: α = 0.01(incidental difference),

**Table IV. Observed genotypes at locus *Dia–B* and number of investigated beech per stand.**

| Stand | Genotype frequency (*Dia–B*) | | | | | | | *W* |
|---|---|---|---|---|---|---|---|---|
| | *N* | $B_1B_1$ | $B_1B_2$ | $B_1B_3$ | $B_2B_2$ | $B_2B_3$ | $B_3B_3$ | |
| F | 151 | 0 | 3 | 0 | 129 | 18 | 1 | ** |
| G | 100 | 0 | 0 | 0 | 92 | 8 | 0 | ** |
| H | 99 | 0 | 0 | 0 | 87 | 12 | 0 | ** |
| I | 99 | 0 | 1 | 0 | 76 | 20 | 2 | ** |
| J | 100 | 0 | 0 | 0 | 82 | 18 | 0 | ** |
| K | 100 | 0 | 0 | 1 | 89 | 10 | 0 | ** |
| L | 100 | 0 | 0 | 0 | 93 | 6 | 1 | ** |
| M | 100 | 0 | 0 | 0 | 94 | 6 | 0 | ** |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: ** : $\alpha = 0.05$ (incidental difference), *: $\alpha = 0.01$ (incidental difference), - : $\alpha < 0.01$ (non incidental difference).

**Table VI. Observed genotypes at locus *Lap–A* and number of investigated beech per stand.**

| Stand | Genotypes frequency (*Lap–A*) | | | | | | | | | | | *W* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | $A_1A_1$ | $A_1A_2$ | $A_1A_3$ | $A_1A_4$ | $A_2A_2$ | $A_2A_3$ | $A_2A_4$ | $A_3A_3$ | $A_3A_4$ | $A_4A_4$ | |
| G | 100 | 1 | 6 | 4 | 2 | 13 | 15 | 22 | 16 | 15 | 6 | ** |
| H | 94 | 0 | 0 | 0 | 4 | 24 | 7 | 27 | 11 | 5 | 16 | – |
| I | 100 | 0 | 3 | 2 | 3 | 17 | 18 | 18 | 10 | 13 | 16 | ** |
| J | 100 | 0 | 3 | 3 | 1 | 12 | 11 | 19 | 21 | 13 | 17 | – |
| K | 100 | 1 | 10 | 4 | 3 | 21 | 15 | 12 | 15 | 11 | 8 | * |
| L | 100 | 1 | 1 | 1 | 0 | 18 | 8 | 29 | 14 | 9 | 19 | – |
| M | 100 | 2 | 5 | 0 | 6 | 23 | 12 | 19 | 11 | 11 | 11 | – |
| N | 99 | 3 | 1 | 3 | 1 | 11 | 9 | 16 | 20 | 17 | 18 | – |
| O | 93 | 0 | 1 | 5 | 1 | 19 | 6 | 31 | 11 | 10 | 9 | – |
| P | 96 | 0 | 0 | 3 | 0 | 13 | 14 | 27 | 11 | 14 | 14 | ** |
| Q | 99 | 0 | 1 | 1 | 4 | 12 | 22 | 27 | 9 | 12 | 11 | ** |
| R | 100 | 0 | 0 | 0 | 0 | 16 | 15 | 34 | 9 | 5 | 11 | ** |
| S | 96 | 0 | 0 | 1 | 1 | 19 | 9 | 26 | 14 | 9 | 17 | – |

*N*: number of trees investigated per stand, *W*: significance level of deviation from Hardy-Weinberg, distribution: ** : $\alpha = 0.05$ (incidental difference), * : $\alpha = 0.01$(incidental difference), - : $\alpha < 0.01$(non incidental difference).

**Table VII. Observed genotypes at locus *Pod–D* and number of investigated beech per stand.**

| Stand | Genotypes frequency (*Pod–D*) | | | | | | | *W* |
|---|---|---|---|---|---|---|---|---|
| | *N* | *D* | $D_1D_2$ | $D_2D_2$ | $D_2D_3$ | $D_3D_3$ | $D_1D_1$ | |
| G | 100 | 5 | 17 | 0 | 65 | 11 | 2 | * |
| H | 99 | 3 | 14 | 2 | 65 | 14 | 1 | ** |
| I | 100 | 1 | 14 | 2 | 59 | 20 | 4 | ** |
| J | 100 | 5 | 12 | 3 | 70 | 8 | 2 | * |
| K | 100 | 1 | 14 | 5 | 55 | 25 | 0 | ** |
| L | 100 | 2 | 13 | 7 | 60 | 16 | 2 | * |
| M | 100 | 4 | 16 | 1 | 60 | 18 | 1 | ** |

*N*: number of trees investigated per stand, *W*:significance level of deviation from Hardy-Weinberg, distribution: **: $\alpha = 0.05$ (incidental difference), - : $\alpha < 0.01$ (non incidental difference).