GENETIC VARIABILITY OF TWO POPULATIONS OF THE ECTOMYCORRHIZAL FUNGUS *XEROCOMUS CHRYSENTERON* ASSOCIATED WITH EUROPEAN BEECH (*FAGUS SYLVATICA* L.)

Constanze von Meltzer & Gunter M. Rothe

Institut für Allgemeine Botanik, Fachbereich Biologie, Johannes Gutenberg-Universität, D-55099 Mainz, Germany

Correspondence: Dr. Gunter M. Rothe, Department of General Botany, Faculty of Biology, Johannes Gutenberg-University, D-55099 Mainz, Germany (tel.: +49-6131-3922535; Fax: +49-6131-3925661; email: rothe@mail.uni-mainz.de)

Received August 16, 1999; accepted February 2, 2000

ABSTRACT

The intra- and interpopulation genetic variability of the ectomycorrhizal fungus *Xerocomus chrysenteron* (Bull. Ex. St. Amans) Quél, infecting European beech (*Fagus sylvatica* L.), was studied in two populations in central Germany (Bingen and Heppenheim). The allele and genotype frequencies of the polymorphic enzyme loci *Acp–B, Dia–B, Dia–C, G6pdh–A, Lap–B, Mdh–C, Me–B, Pep1–B* and *Pep2–B* served as genetic markers. The total number of alleles of the nine gene loci was 23 (allele/polymorphic locus = 2.4). The alleles 1 of the loci *Me–B* and *Dia–B* were observed only in one of the populations (Heppenheim). The average number of alleles per polymorphic locus was a little higher in one stand (Heppenheim: 2.55) than in the other (Bingen: 2.33). The number of effective alleles was calculated to be n_e = 1.56 (Heppenheim) and n_e = 1.53 (Bingen) in both populations. The genotypes *Dia–B* 1/1, *G6pdh–A* 1/2, *Lap–B* 1/3, *Mdh–C* 1/2 and *Me–B* 1/1, 1/2, 1/3 were found only in one population (Heppenheim), while they were missing in Bingen. On the other hand, the Bingen population showed the genotypes *G6pdh–A* 1/1 and *Lap–B* 1/1 which were missing in the Heppenheim population. Relative allelic frequencies varied little (3 to 5 %) at the loci *Dia–C, G6pdh–A, Lap–B, Mdh–C, Pep1–B* and *Pep2–B* whereas the frequency differences of the alleles 1 and 2 at the locus *Acp–B* were considerable in both populations (allele 1: 46 % (Heppenheim), 82 % (Bingen)). As a consequence, a low genetic distance (D = 3 %, d_o = 0.9 %) was observed among the two populations studied. Also, the average heterozygosity was not significantly different in both populations (H_E = 33 %). An excess of heterozygous genotypes was observed in both populations at the loci *Me–B* and *Pep2–B*. The total genetic diversity was H_T = 0.3466, the genetic diversity within populations was H_S = 0.3381 and the amount of genetic differentiation of the total diversity was G_{ST} = 2.5 %. These data are compared with known population genetic data of European beech and similarities and differences are discussed.

Key words: Basidiomycetes, ectomycorrhiza, European beech, *Fagus sylvatica,* isozymes, population genetics, symbiosis, *Xerocomus chrysenteron*

INTRODUCTION

European beech (*Fagus sylvatica* L.) lives in close symbiosis with some 30 root ectomycorrhizal fungi (AGERER 1988–1998; BRAND 1989, 1991). The genetic variability of beech populations has been investigated intensively (MÜLLER-STARCK & STARKE 1993; KONNERT 1995; LEONARDI & MENOZZI 1995; LÖCHELT & FRANKE 1995; HATTEMER & ZIEHE 1996), but the intra- and interpopulation genetic variability of their fungal partners is completely unknown. From other mycorrhizal fungi, it is known that different isolates of a particular species can exhibit great variation in ecological and physiological functions, such as in the synthesis of enzymes (LUNDEBERG 1970), in the uptake of mineral nutrients (LITTKE *et al.* 1984), and in the symbiotic effectiveness (MOLINA 1979; MARX 1981). However, the genetic basis of such intraspecific variation has not been investigated. So far, the ectomycorrhizal fungus *Suillus tomentosus* (Kauffm.) Singer, Suell & Dick, occurring in the boreal forests of Canada, is the only one in which the intra- and interpopulation genetic variability has been studied, using cluster analysis (ZHU *et al.* 1988). In association with different tree species, the interpopulation genetic variability of the fungus was larger than its intrapopulation genetic variability (ZHU *et al.* 1988).

The aim of this investigation was to compare the inter- and intrapopulation genetic variability of the ectomycorrhizal fungus *Xerocomus chrysenteron* (Bull. Ex. St. Amans) Quél and to relate the data obtained to the genetic structure of its host European beech (*Fagus sylvatica* L.).

Table 1. Number of extract application and buffer systems used to separate enzymes of *Xerocomus chrysenteron* in cellulose acetate electrophoresis.

Enzyme system	Number of extract applications*	Buffer system
Acid phosphatase	2	G
Diaphorase	2	L
Glucose-6-phosphate dehydrogenase	2	T–C
Leucine aminopeptidase	1	L
Malate dehydrogenase	2	L
Malic enzyme	4	L
Peptidase 1 (Gly-Leu as substrate)	1	T–G
Peptidase 2 (Leu-Gly-Gly as substrate)	1	T–G

*) one application corresponds to approximately 0.25 µl

Buffer G: Tris-maleic acid, pH 7.8 (RICHARDSON *et al.* 1986): 100 mM Tris, 40 mM maleic acid

Buffer L: Tris-maleic acid EDTA-$MgCl_2$, pH 7.8 (RICHARDSON *et al.* 1986): 50 mM tris, 20 mM maleic acid, 1 mM EDTA- 1 mM Mg Cl_2-$6H_2O$

Buffer T-C: Tris citrate, pH 7.0 (SOLTIS & SOLTIS 1990): 135 mM Tris, 43 mM citric acid-monohydrate

Buffer T-G: Tris-glycine, pH 8.5 (HEBERT & BEATON 1993): 25 mM Tris, 192 mM glycine (diluted 1 in 10 for use)

diphenyltetrazolium bromide (10 $mg \cdot ml^{-1}$) (cf. HARRIS & HOPKINSON 1976) (Figure 2).

Peptidase 1 (EC 3.4.11.*) was stained with 2 ml 20 mM phosphate buffer, pH 7.5, 4 drops of peroxidase (200 $Units \cdot mg^{-1}$, 5 $mg \cdot ml^{-1}$; Boehringer, Mannheim), 8 drops of o-dianisidine-2HCl (4 $mg \cdot ml^{-1}$), 2 drops of $MgCl_2$-$6H_2O$ (20 $mg \cdot ml^{-1}$), 8 drops of the dipeptide glycine-leucine (5 $mg \cdot ml^{-1}$) and 4 drops of amino acid oxidase (0.55 $units \cdot mg^{-1}$; 20 $mg \cdot ml^{-1}$; Sigma, München) (HEBERT & BEATON 1993) (Figure 7).

Peptidase 2 (EC 3.4.11.*) was stained as for peptidase 1 but using 8 drops of the tripepetide leucine-glycine-glycine (5 mg . ml^{-1}) instead of the dipeptide (HEBERT & BEATON 1993) (Figure 8).

Acid phosphatase (EC 3.1.3.2) (Figure 1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (Figure 3), leucine aminopeptidase (EC 3.4.11.1) (Figure 4), malate dehydrogenase (EC 1.1.1.37) (Figure 5) and malic enzyme (EC 1.1.1.40) (Figure 6) were visualized as previously described (FIEDLER & ROTHE 1999).

Exploitation of electrophoretic data

Because of the dikaryotic state of the hyphae, electrophoretic enzyme patterns were translated into genotypes and alleles at gene loci according to known enzymatic structures of diploid material (ZHU *et al.* 1988; SOLTIS & SOLTIS 1990; ROTHE 1994). Definition and utilization of mathematical formulae were as proposed by WRIGHT (1978), ROTHE (1994) and YEH *et al.* (1998). The software POPGENE Version 1.31 (YEH *et al.* 1998) with the following definitions and formulae was applied: genetic identity = I, genetic distances = D and d_0 (Table 9), heterozygosity = H_L and H_E (Table 10), genetic diversities = H_S, H_T, D_{ST} and G_{ST} (Table 11), fixation index = F_{IS} (Table 12). Differences in relative frequencies (p %) of genotypes at a significance level of $\alpha = 5$ % were calculated by using the formula $\alpha_{(p=5\%)} = 1.96 \cdot v$ with $v_{p\%} = \pm((p\,(100 - p) / N)^{1/2}$ with N: number of samples per population (B. Thiebaut, personal communication).

RESULTS

Investigated enzyme loci

Isoenzyme banding patterns were interpreted assuming that the mycelia of *Xerocomus chrysenteron* are dikaryotic because in the ectomycorrhiza the basidiomycete forms a clamp mycelium (BRAND 1991). The clamp mycelium represents a dikaryotic state which is genetically equivalent of a diploid (ESSER 1986). Therefore genotype and allele frequencies at nine different enzyme gene loci (Table 2) were interpreted as for diploid organisms. Acid phosphatase, leucine aminopeptidase, malic enzyme and peptidase 2 are assumed to be monomeric enzymes, whereas malate dehydrogenase, diaphorase and peptidase 1 are thought to be dimeric enzymes (cf. ZHU *et al.* 1988) (Figures 1–8). As an example of a monomeric enzyme with two alleles, glucose-6-phosphate dehydrogenase can be taken (Figure 3), while an example of a dimeric enzyme with two alleles is peptidase 1 (Figure 7). The monomeric enzyme system shows two allelic variants when heterozygous and, one of the two when homozygous (Figure 3) while the dimeric system shows three electrophoretic variants when heterozygous and one, either the slowest or the fastest migrating form, when homozygous (Figure 7).

Population genetic measures

Polymorphic loci and effective number of alleles

The nine polymorphic gene loci investigated showed a total of 23 alleles, but two of these were found only in one of the investigated beech stands (Heppenheim), namely the alleles *Dia–*B_1 and *Me–*B_1 (Table 3). Therefore, the average number of alleles per polymorphic

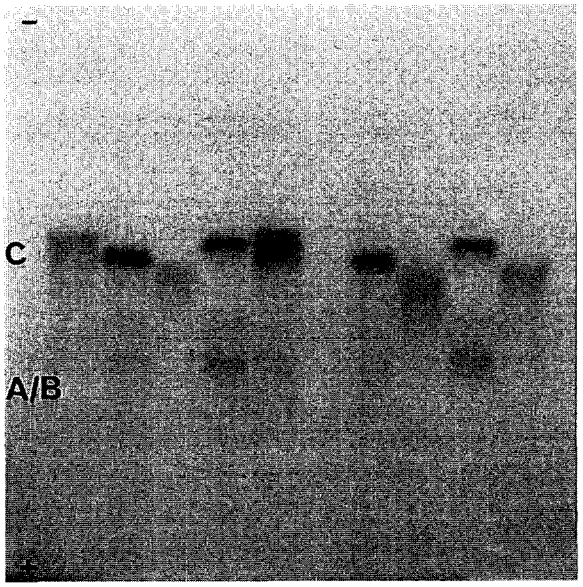

Figure 5. Zymogram of malate dehydrogenase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Mdh-C* genotypes 3/3, 2/2, 1/1, 3/3, 2/3, control, 2/2, 1/2, 3/3, 1/1. Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter).

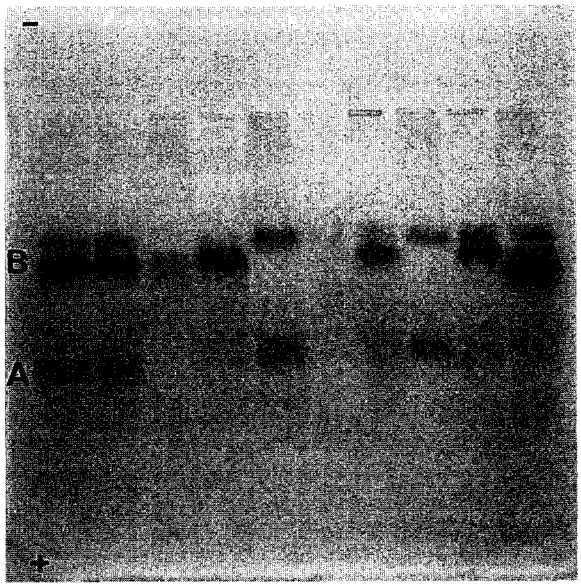

Figure 6. Zymogram of malic enzyme allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Me-B* genotypes 2/3, 2/3, 2/2, 2/2, 3/3, control, 2/3, 3/3, 2/3, 1/3. Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter).

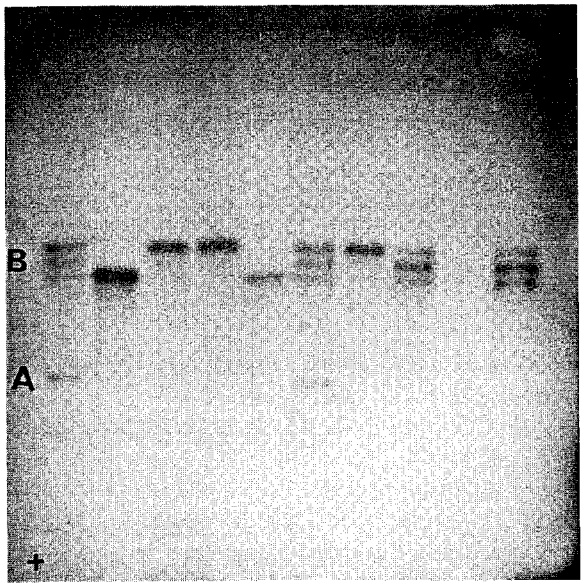

Figure 7. Zymogram of peptidase 1 allozymes (substrate: glycine-leucine) of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Pep 1-B* genotypes 1/2, 1/1, 2/2, 2/2, 1/1, 1/2, 2/2, 1/2, control, 1/2. Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter).

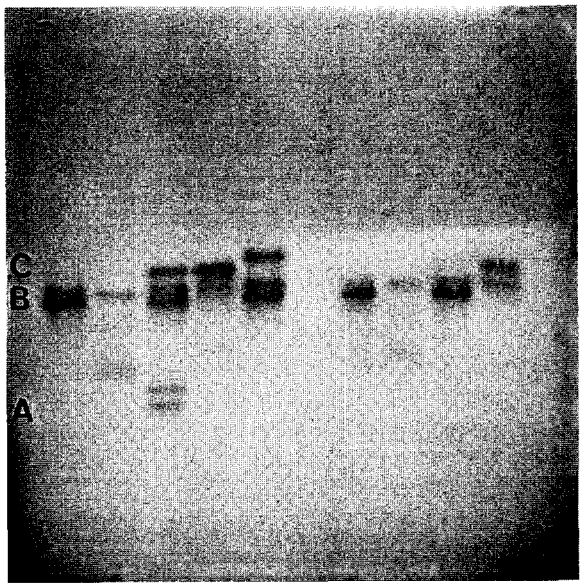

Figure 8: Zymogram of peptidase 2 allozymes (substrate: leucine-glycine-glycine) of mycorrhizae of *Fagus sylvatica /Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Pep 2-B* genotypes 1/2, 2/2, 1/2, 2/2, 1/2, control, 1/1, 2/2, 1/2, 2/2. Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter).

Table 4. Relative frequencies (%) genotypes in the *Xerocomus chrysenteron* population from Bingen.

Genotype	Genotype frequencies (%) at locus								
	Acp–B	*Dia–B*	*Dia–C*	*G6pdh–A*	*Lap–B*	*Mdh–C*	*Me–B*	*Pep1–B*	*Pep2–B*
1/1	75.93[1]	0	83.33	3.70	3.70	3.70	0	16.67	14.81
1/2	11.11	0	7.41	0	0	0	0	37.04	62.96
1/3	–	0	–	–	0	0	0	–	–
1/4	–	–	–	–	0	–	–	–	22.22
2/2	12.96[1]	74.07	9.26	96.30	0	72.22	12.96	46.30	–
2/3	–	9.26	–	–	5.56	16.67	72.22	–	–
2/4	–	–	–	–	0	–	–	–	–
3/3	–	16.67	–	–	74.07	7.41	14.81	–	–
3/4	–	–	–	–	12.96	–	–	–	–
4/4	–	–	–	–	3.70	–	–	–	–

0: Genotype not detected, no number: genotype not existing; [1)] frequency significantly different from that at stand Heppenheim at a significance level of $\alpha = 5\%$, investigated samples $N = 54$.

Table 5. Relative frequencies (%) of genotypes in the *Xerocomus chrysenteron* population from Heppenheim

Genotype	Genotype frequencies (%) at locus								
	Acp–B	*Dia–B*	*Dia–C*	*G6pdh–A*	*Lap–B*	*Mdh–C*	*Me–B*	*Pep1–B*	*Pep2–B*
1/1	37.70[1]	1.64	80.33	0	0	1.64	1.64	6.56	11.48
1/2	16.39	0	8.20	11.48	0	3.28	1.64	45.90	63.93
1/3	–	0	–	–	6.56	0	8.20	–	–
1/4	–	–	–	–	0	–	–	–	–
2/2	45.90[1]	75.41	11.48	88.52	0	73.77	3.28	47.54	24.95
2/3	–	19.67	–	–	4.92	13.11	62.30	–	–
2/4	–	–	–	–	0	–	–	–	–
3/3	–	3.28	–	–	77.05	8.20	22.95	–	–
3/4	–	–	–	–	11.48	–	–	–	–
4/4	–	–	–	–	0	–	–	–	–

0: Genotype not detected, no number: genotype not existing; [1)] frequency significantly different from that at stand Heppenheim at a significance level of $\alpha = 5\%$, investigated samples $N = 61$.

Bingen and Heppenheim population (Tables 4 and 5).

At locus *Dia–C* there were three genotypes (Figure 2). The most prominent one in both populations was type 1/1 (80.33 % and 83.33 %); the two remaining types 1/2 and 2/2 were equally frequent in both populations (1/2: 7.41 % and 8.02 %; 2/2: 9.26 % and 11.48 %) (Tables 4 and 5).

Locus *G6pdh–A* showed three genotypes (Figure 3). The type 2/2 was most frequent in both populations (96.30 % and 88.52 %, respectively) (Tables 4 and 5). Genotype 1/1 was exclusively found in the population from Bingen (3.70 %), while the genotype 1/2 appeared only in the population from Heppenheim (11.48 %) (Tables 4 and 5).

At locus *Lap–B* six genotypes were observed (Figure 4). Of these, the type 3/3 was most prominent in both populations (74.07 % and 77.05 %). The genotypes 1/1 (3.70 %) and 4/4 (3.70 %) appeared only in the Bingen population, while the type 1/3 existed only in the Heppenheim population (6.56 %). The genotypes 2/3 (5.56 % and 4.92 %) and 3/4 (12.96 % and 11.48 %) were equally frequent in both populations (Tables 4 and 5).

Locus *Mdh–C* comprises five genotypes (Figure 5). Of these, the type 2/2 was most prominent in both populations (72.22 % and 73.77 %). The type 1/2 exclusively appeared in the Heppenheim population (3.28 %). The genotypes 1/1 (3.70 %), 2/3 (16.67 %) and 3/3 (7.41 %) were equally frequent in the Bingen population and the Heppenheim population (1.64 %,

showed unique genotypes at several loci (*Dia–B* 1/1, *G6pdh–A* 1/1 and 1/2, *Lap–B* 1/1, 1/3 and 4/4, *Mdh–C* 1/2, *Me–B* 1/1, 1/2 and 1/3). If the corresponding enzymes have different physicochemical properties or are localized at different cell compartments, their presence or absence would indicate metabolic differences in both species with respect to glycolysis (G6pdh), malic acid-metabolism (Mdh, Me), fatty acid-metabolism (Dia) and peptide cleavage (Lap). The soil at both stands is different and the two populations could have adapted to this situation. However, a limited gene flow between both populations of *X. chrysenteron* is also possible due to the natural borders of the river Rhine.

The common genetic properties of European beech and *X. chrysenteron* are most possibly the result of a co-evolution which started after *Fagus sylvatica* occupied its natural range after the last glaciation some 11 000 years ago. This opinion is not in contradiction to the result that Canadian populations of *Suillus tomentosus* showed larger interpopulation genetic variabilities than intrapopulation genetic variabilities when each population infected different species of trees (ZHU *et al.* 1988).

ACKNOWLEDGEMENT

This work was supported financially by the Deutsche Forschungsgemeinschaft.

REFERENCES

AGERER, R. 1988–1998: Color Atlas of Ectomycorrhizae, Einhorn-Verlag, Schwäbisch-Gmünd, 140 pp.

BELLETI, P. & LANTERI, S. 1996: Allozyme variation among European beech (*Fagus sylvatica* L.) stands in Piedmont, North-Western Italy. *Silvae Genetica* **45**: 33–37.

BRAND, F. 1989: Studies on ectomycorrhizae. XXI. Beech ectomycorrhizae and rhizomorphs of *Xerocomus chrysenteron* (*Boletales*). *Nova Hedwigia* **48**: 469–483.

BRAND, F. 1991: Ektomykorrhizen an *Fagus sylvatica*. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. IHW–Verlag, Eching, 229 pp.

DÄHNE, J., KLINGELHÖFER, D., OTT, M. & ROTHE, G. M. 1995: Liming induced stimulation of the amino acid metabolism in mycorrhizal roots of Norway spruce (*Picea abies* [L.] Karst.). *Plant and Soil* **173**: 67–77.

ESSER, K. 1986: Kryptogamen, Springer-Verlag, Berlin, Heidelberg, Germany, 566 pp.

FIEDLER, O. & ROTHE, G. M. 1999: Cellulose acetate electrophoresis of the beech ectomycorrhiza *Fagus sylvatica* – *Xerocomus chrysenteron*. In: From Symbiosis to Eukaryotism, Endocytobiology VII. (eds. E. Wagner, J. Norman, H. Greppin, J. H. P. Hackstein, R. G. Herrmann, K. V. Kowallik, H. E. A. Schenk, J. Seckbach). Vol 1. University of Geneva, 1999, pp. 253– 269.

GREGORIUS, H. R. 1974: Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. *Silvae Genetica* **23** (1–3): 22–27.

HARRIS, H. & HOPKINSON, D. A. 1976: Handbook of enzyme electrophoresis in human genetics, American Elsevier, New York, 4–1 pp.

HATTEMER, H. H. & ZIEHE, M. 1996: An attempt to infer on the origin of a Beech (*Fagus sylvatica* L.) stand in Rheinland-Pfalz (Germany). *Silvae Genetica* **45**: 276–283.

HEBERT, P. D. N. & BEATON, M. J. 1993: Methodologies for allozyme analysis using cellulose acetate electrophoresis. A practical handbook. An educational service of Helena Laboratories, Beaumont, Texas, U. S. A, 32 pp.

KONNERT, M. 1995: Investigations on the genetic variation of beech (*Fagus sylvatica* L.) in Bavaria. *Silvae Genetica* **44**: 346–351.

LEONARDI, S. & MENOZZI, P. 1995: Genetic variability of *Fagus sylvatica* L. in Italy: the role of postglacial recolonization. *Heredity* **75**: 35–44.

LITTKE, W. R., BLEDSOE, C. S. & EDMONDS, R. L. 1984: Nitrogen uptake and growth in vitro by *Hebeloma crustuliniforme* and other Northwest mycorrhizal fungi. *Can. J. Bot.* **62**: 647–652.

LÖCHELT, S. & FRANKE, A. 1995: Bestimmung der genetischen Konstitution von Buchen-Beständen (*Fagus sylvatica* L.) entlang eines Höhentransektes von Freiburg auf den Schauinsland. *Silvae Genetica* **44**: 312–318.

LUNDEBERG, G. 1970: Utilization of various nitrogen sources, in particular bound soil nitrogen by mycorrhizal fungi. *Stud. For. Suec.* **79**: 1–95.

MARX, D.H. 1981: Variability in ectomycorrhizal development and growth among isolates of *Pisolithus tinctorius* as affected by source, age, and reisolation. *Can. J. For. Res.* **11**: 168–174.

MOLINA, R. 1979: Ectomycorrhizal inoculations of containerized Douglas-fir and lodgepole pine seedlings with six isolates of *Pisolithus tinctorius*. *For. Sci.* **25**: 585–590.

MÜLLER-STARCK, G. 1991: Survey of genetic variation as inferred from enzyme gene marker. In: Genetic variation in European populations of forest trees. (eds. G. Müller-Starck and M. Ziehe). pp 20–37. Sauerländer's Verlag, Frankfurt am Main.

MÜLLER-STARCK, G. & STARKE, R. 1993: Inheritance of isoenzymes in European beech (*Fagus sylvatica* L.). *Journal of Heredity* **84**: 291–296.

NEI, M. 1972: Genetic distance between populations. *The American Naturalist* **106** (949): 283–292.

NEI, M. 1973: Analysis of gene diversity in subdivided populations. *Proc. Acd. Sci.* USA **70**: 3321–3323.

RICHARDSON, B. J., BAVERSTOCK, P. R. & ADAMS, M. 1986: Allozyme electrophoresis. A handbook for animal systematics and population studies, Academic Press, Sydney, Australia, 410 pp.

ROTHE, G. M. 1994: Electrophoresis of enzymes, Springer Verlag, Berlin, Heidelberg, Germany, 307 pp.

SANDER, T., ROTHE, G. M., WEISGERBER, H. & JANßEN, A. 1999: Analyse der genetischen Variabilität und Diversität von Rotbuchen (*Fagus sylvatica* L.) – Beständen in Hessen. Submitted for publication.

SOLTIS, D. E. & SOLTIS, P. S. 1990: Isozymes in Plant Biology. Chapman and Hall Ltd., London, UK, 268 pp.