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

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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.).

MATERIAL AND METHODS

Stand characteristics

Fine roots (d ≤ 2 mm) which were infected at their tips with *Xerocomus chrysenteron* (Bull. Ex. St. Amans) Quél were sampled in two beech (*Fagus sylvatica* L.) stands in Central Germany.

One of the beech stands is seven hectars large and located at the Odenwald hills, about 36 km south of Frankfurt at Main, in the forest district of Heppenheim, logging area Kirschhausen, compartment 101. The beech trees are approximately 100 years old (1999). They grow on a moderate to steep, north-west inclined, slope at 305 to 350 m above sea level on a loess loam, above granite. The moderately well drained site was not limed. The last selective logging was performed in winter 1994/95.

The other beech stand is eight hectars large and located at the Hunsrück hills, about 45 km west of Frankfurt at Main, in the forest district of Bingen, logging area Jägerhaus, compartment 56. The approximately 100 year old (1999) beech trees grow on a moderate, south easternly inclined, slope at 465 to 515 m above sea level on a sandy loam above quartzite. The moderately well drained site was limed in 1989 with 3 tons per hectar of magnesium rich limestone (50 % $CaCO_3$, 40% $MgCO_3$). The last selective logging was performed in winter 1996.

Root sampling

In the beech wood of forest district Heppenheim, a total of 61 root samples (individuals) were taken from the end of April to the beginning of June 1998 and analyzed for isozyme patterns. In the beech wood of forest district Bingen, a total of 54 root samples (individuals) were taken between mid of September and end of October 1998 and analyzed thereafter. About 1.5 to 2 m apart from the base of a beech, a soil square of about 1 m^2 was freed from the litter. The upper soil was loosened by hand or using a spatula and investigated for the occurrence of fine roots. Then, fine roots were dug out to a soil depth of approximately 5 cm, freed from coarse soil particles and inspected for white mycorrhizas of the type beech-*Xerocomus chrysenteron*. If root tips were infected with this fungus, the fine root (d ≤ 2 mm) to which it belonged, was followed until free of this and any other fungus. Each root sample was put into a separate plastic bag, numbered and stored on ice. Within the next four days, the roots were transferred to a Petri dish with ice water, cleaned with a fine brush and finely selected for infections with *X. chrysenteron* and, identified under a stereo microscope at a 25-fold magnification according to the morphological criteria described by BRAND (1989) and AGERER (1988–1998). The very same clusters of one sample were then separated into root tips infected with the dikaryotic *X. chrysenteron* and non-infected proximal parts (d = 1 to 2 mm) (cf. DÄHNE *et al.* 1995). Mycorrhizal root treelets having a fresh weight of at least 50 mg were put in a 1.5 ml Eppendorf-safe-lock-tube, frozen for 10 sec in liquid nitrogen and stored at –80 °C. Non-mycorrhizal roots of the same root cluster were treated similarily.

Enzyme extraction

A 50 mg quantity of either mycorrhizal or non-mycorrhizal roots was homogenized with 100 µl extraction medium and 7.5 mg of wet PVPP (polyclar AT) as described by FIEDLER and ROTHE (1999). Afterwards, the slurry was thoroughly mixed with a spatula and stored on ice for 10 min. The extraction medium consisted of (mg·100 ml^{-1}) of 100 mM Na-phosphate buffer, pH 7.0): cysteine (30), 2-mercaptobenzothiazole (3.3), Na-metabilsulfite (95), EDTA-Na_2 (186), $MgCl_2$-$6H_2O$ (102), NADP (39.2), NAD (35.8), sucrose (14 000), bovine serum albumine (500) and Tween^R 80 (500) (FIEDLER & ROTHE 1999). The homogenized sample was centrifuged for 25 min at 4 °C and 5 000 rpm (Minifuge GL, Heraeus Christ, Osterode). Aliquots of 20 µl of the supernatand were transferred into 0.5 ml Eppendorf-tubes and stored at –30 °C.

Cellulose acetate electrophoresis

Electrophoresis was performed on 76 × 76 mm cellulose acetate plates (Titan^{R}III, Helena Laboratories, Helena Diagnostika, Hartheim), as described earlier (FIEDLER & ROTHE 1999), but using the sample amounts and buffer systems given in Table 1.

Enzyme visualization

After electrophoresis cellulose acetate plates were placed, mylar side down, on a glass plate. A volume of 2 ml of melted agar (20 mg·ml^{-1}, 60 °C) was added to the staining solution and the mixture (4 to 5 ml per plate) poured over the gel plates. After the agar had set, the plates were incubated in the dark at 37 °C until enzyme bands were visible.

Diaphorase (Cytochrome b5 reductase, EC 1.6.2.2) was stained with a mixture of 1 ml of 100 mM Tris-HCl, pH 8.5, 1.5 ml NADH (3 mg·ml^{-1}), 5 drops of dichlorophenolindophenole (DCPIP) (3 mg·ml^{-1}) and 5 drops of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-

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

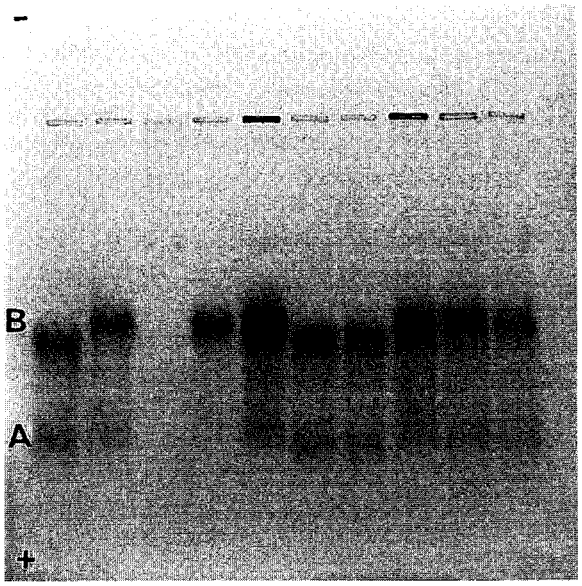

Figure 1. Zymogram of acid phosphatase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Acp-B* genotypes 1/1, 2/2, control, 2/2, 1/2, 1/1, 1/1, 1/2, 2/2, 2/2. Control: Extract of non-mycorrhizal beech fine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

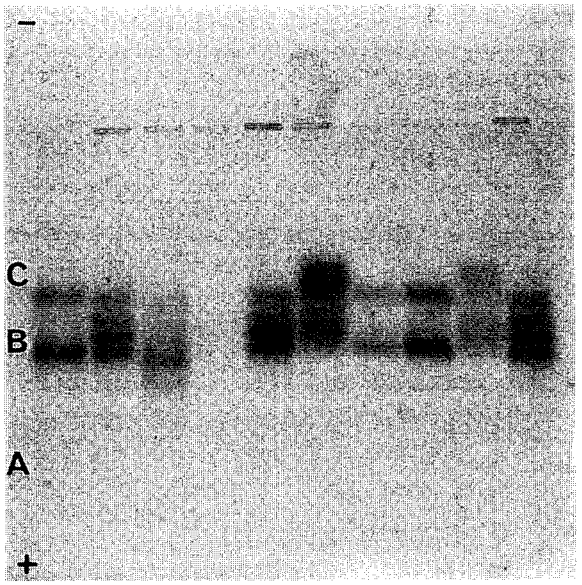

Figure 2. Zymogram of diaphorase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Dia-B* genotypes: 2/2, 2/3, 1/1, control, 2/3, 2/3, 2/2, 2/2, 3/3, 2/3 and *Dia-C* genotypes: 1/1, 1/1, 1/1, control, 1/1,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

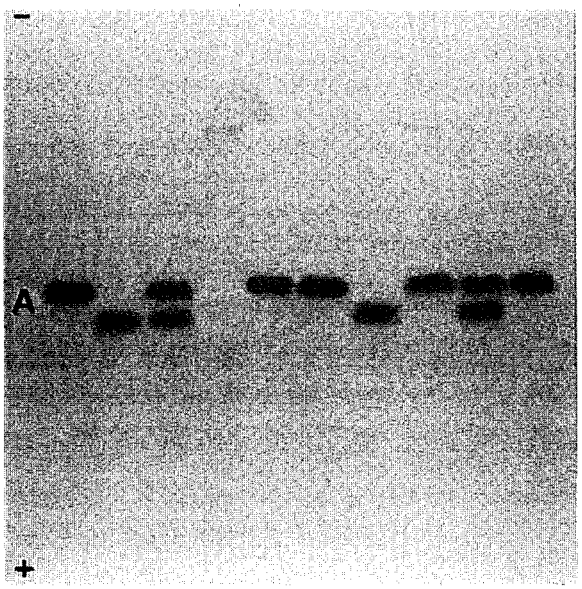

Figure 3. Zymogram of glucose-6-phosphate dehydrogenase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* taken from two beech stands in the forest districts of Bingen (B) and Heppenheim (H). From left to right: *G6pdh-A* genotypes 2/2 (H), 1/1 (B), 1/2 (H), control, 2/2 (B), 2/2 (H), 1/1 (B), 2/2 (B), 1/2 (H), 2/2 (H). Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter) from Heppenheim..

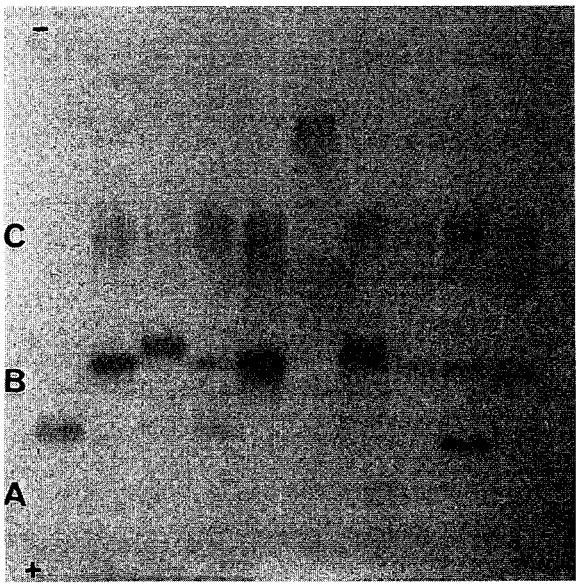

Figure 4. Zymogram of leucine aminopeptidase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* taken from two beech stands in the forest districts of Bingen (B) and Heppenheim (H). From left to right: *Lap-B* genotypes 1/1 (B), 3/3 (B), 4/4 (B), 1/3 (H), 2/3 (B), control, 3/4 (H), 3/3 (B), 1/3 (H), 3/3 (B). Control: Extract of non-mycorrhizal beech fine roots (1 to 2 mm in diameter) from Bingen.

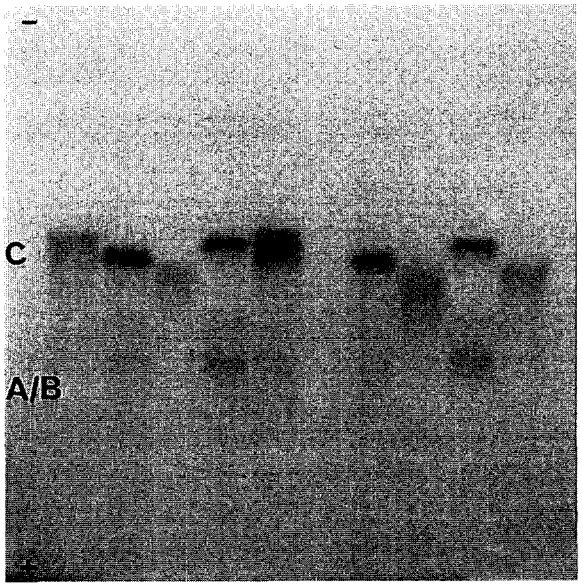

Figure 5. Zymogram of malate dehydrogenase allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Mdh-C* genotypes 3/3, 2/2, 1/1, 3/3, 2/3, control, 2/2, 1/2, 3/3, 1/1. Control: Extract of non-mycorrhizal beech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

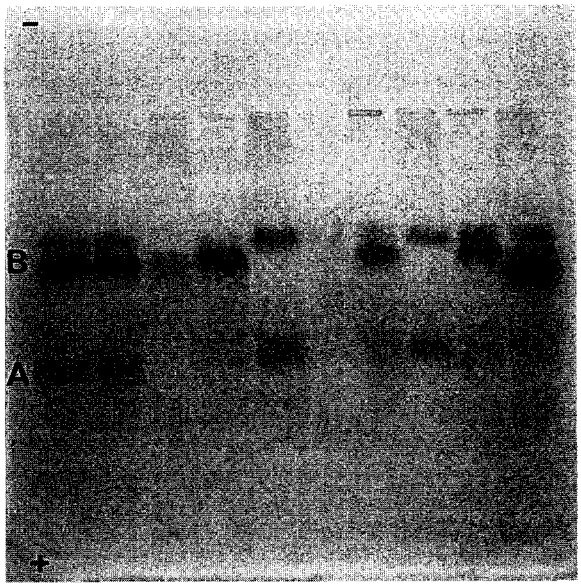

Figure 6. Zymogram of malic enzyme allozymes of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Me-B* genotypes 2/3, 2/3, 2/2, 2/2, 3/3, control, 2/3, 3/3, 2/3, 1/3. Control: Extract of non-mycorrhizal beech fine roots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

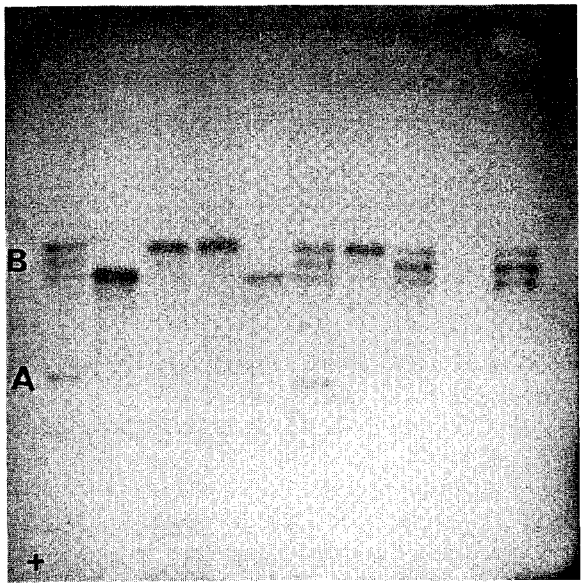

Figure 7. Zymogram of peptidase 1 allozymes (substrate: glycine-leucine) of mycorrhizae of *Fagus sylvatica/Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Pep 1-B* genotypes 1/2, 1/1, 2/2, 2/2, 1/1, 1/2, 2/2, 1/2, control, 1/2. Control: Extract of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

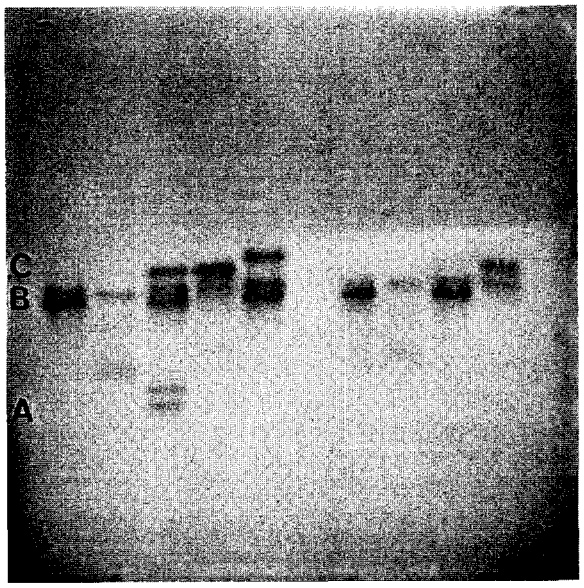

Figure 8: Zymogram of peptidase 2 allozymes (substrate: leucine-glycine-glycine) of mycorrhizae of *Fagus sylvatica /Xerocomus chrysenteron* from a beech stand in the forest district of Heppenheim. From left to right: *Pep 2-B* genotypes 1/2, 2/2, 1/2, 2/2, 1/2, control, 1/1, 2/2, 1/2, 2/2. Control: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

Table 2. Enzyme systems, corresponding enzyme loci, scored enyzme loci, number of subunits per enyzme and alleles per enyzme locus investigated in *Xerocomus chrysenteron*.

Enzyme system	Loci	Scored loci	Subunits per enzyme	Alleles per locus
Acid phosphatase	*Acp–A, –B*	B	1	2
Diaphorase	*Dia–A, –B, –C*	B	2	3
		C	2	2
Glucose-6-phosphate dehydrogenase	*G6pdh–A*	A	1	2
Leucine aminopeptidase	*Lap–A, –B, –C*	B	1	4
Malate dehydrogenase	*Mdh–A/B?, –C*	C	2	3
Malic enzyme	*Me–A, –B*	B	1	3
Peptidase 1	*Pep1–A, –B*	B	2	2
Peptidase 2	*Pep2–A, –B, –C*	B	1	2

Table 3. Number of alleles and effective number of alleles (*n*)of the nine enzyme loci investigated in the two *Xerocomus chrysenteron* populations Bingen and Heppenheim.

Enzyme locus	Bingen		Heppenheim	
	Alleles; (number of alleles)	Efective number of alleles (*n*)	Alleles, (number of alleles)	Effective number of alleles (*n*)
Acp–B	B_1, B_2; (2)	1.43	B_1,B_2;(2)	1.99
Dia–B	B_2,B_3; (2)	1.5	B_1,B_2,B_3; (3)	1.34
Dia–C	C_1, C_2;(2)	1.29	C_1, C_2;(2)	1.36
G6pdh–A	A_1, A_2; (2)	1.08	A_1, A_2; (2)	1.12
Lap–B	B_1, B_2, B_3, B_4; (4)	1.41	B_1,B_2,B_3,B_4;(4)	1.27
Mdh–B	B_1, B_2, B_3; (3)	1.48	B_1,B_2,B_3;(3)	1.44
Me–B	B_2, B_3; (2)	2	B_1,B_2,B_3;(3)	2.14
Pep1–B	B_1, B_2; (2)	1.84	B_1,B_2;(2)	1.71
Pep2–B	B_1, B_2; (2)	1.99	B_1,B_2;(2)	1.97
Mean value	2.33	1.53	2.55	1.56

locus was 2.55 for the *X. chrysenteron* population from Heppenheim and 2.33 for the population from Bingen. The effective number of alleles, n_e, which increases linearly with the number of alleles and which may be used to quantify genetic variation between populations was calculated to be n_e = 1.56 for the Heppenheim population, while the effective number of alleles for the Bingen population was estimated to be n_e = 1.53.

Genotype frequencies

The nine polymorphic gene loci investigated showed a total of 34 genotypes in both populations. Therefore, the average number of genotypes per polymorphic locus was 3.22 for the *X. chrysenteron* population from Bingen and 3.66 for the population from Heppenheim. Several genotypes at locus *Dia–B*, *G6pdh–A*, *Lap–B* and *Mdh–C* were found only in the Heppenheim population or in the Bingen population.

Acid phosphatase showed three genotypes at locus *Acp–B* (Figure 1). The homozygous genotype 1/1 was significant more frequent in the Bingen population (75.93 %; Heppenheim: 37.70 %) while the homozygous genotype 2/2 was significant more frequent in the Heppenheim population (45.90 %; Bingen: 12.96 %). The genotype 1/2 was equally frequent in both populations (11.11 % and 16.39 %) (Tables 4 and 5).

At the gene locus *Dia–B* four genotypes were observed (Figure 2). Of these, the type 2/2 was most frequent in both populations (74.07 % and 75.41 %) (Tables 4 and 5). The genotype 1/1 was only observed in the Heppenheim population (1.64 %) (Table 5). The genotypes 2/3 (9.26 % and 19.67 %) and 3/3 (16.67 % and 3.28 %) were not significantly different in the

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 %,

13.11 % and 8.20 %) (Tables 4 and 5).

At locus *Me–B* six genotypes were observed. Figure 6 shows four of them. The most prominent one was type 2/3, in both populations (72.22 %, 62.30 % respectively). The genotypes 1/1 (1.64 %), 1/2 (1.64 %) and 1/3 (8.20 %) were limited to the stand in Heppenheim. In the Bingen stand the types 2/2 (12.96 %) and 3/3 (14.81 %) were not significantly different from the Heppenheim stand (3.28 % and 22.95 %) (Tables 4 and 5).

At locus *Pep1–B* three genotypes were seen (Figure 7). Type 1/1 (16.67 % and 6.56 %), type 1/2 (37.04 % and 45.90 %) and type 2/2 (46.30 % and 47.54 %) were equally frequent in both populations (Tables 4 and 5).

At locus *Pep2–B* three genotypes also appeared (Fig. 8). These were equally frequent in both populations (Tables 4 and 5).

Allele frequencies

Of the 23 alleles detected at the nine polymorphic enzyme loci, two were population specific. The alleles *Dia–B*$_1$ and *Me–B*$_1$ did not occur in the Bingen population, whereas they were observed at low frequencies in the Heppenheim population (*Me–B*$_1$: 6.56 %, *Dia–B*$_1$: 1.64 %; Tables 6 and 7). Larger allele frequency differences were observed at locus *Acp–B* where allele 1 was more prominent in the Bingen population (81.48 %) than in the Heppenheim population (45.90 %), whereas allele 2 occurred at a lower frequency in the Bingen population (18.52 %) than in the Heppenheim population (54.10 %) (Tables 6 and 7). All other investigated alleles appeared at nearly equal frequencies in the two populations in Central Germany.

Hardy-Weinberg distribution of genotypes

The number of observed genotypes at the nine polymorphic enzyme loci corresponds at three loci (*G6pdh–A*, *Lap–B*, *Pep1–B*) in both populations to a Hardy-Weinberg distribution (Table 8). At four loci (*Acp–B*, *Dia–C*, *Mdh–B*, *Me–B*), however, significant deviations were observed in the populations of Bingen and Heppenheim (Table 8). At two gene loci, genotypes were distributed according to Hardy-Weinberg in one population (*Dia–B*: Heppenheim, *Pep2–B*: Bingen) but not in the other one (Table 8).

Genetic identity *I* and genetic distances *D* and d_o

The genetic identity *I* between the two populations Bingen and Heppenheim as defined by NEI (1972) was calculated to be *I* = 97.28 % (Table 9). Correspondingly, the genetic distance according to NEI (1972) is *D* = 2.98 % and that of GREGORIUS (1974) is d_o = 8.60 % (Table 9). The smallest genetic distance was observed at locus *Mdh–B* (*D* = 0.01 %, d_o = 1.41 %), the largest locus *Acp–B* (*D* = 22.33 %, d_o = 35.58 %) (Table 9).

Table 6. Relative allele frequencies (%) of the nine scored enzyme loci of the *Xerocomus chrysenteron* population from Bingen.

Allele number	Allele frequencies (%) at locus								
	Acp–B	*Dia–B*	*Dia–C*	*G6pdh–A*	*Lap–B*	*Mdh–C*	*Me–B*	*Pep1–B*	*Pep2–B*
1	81.48	0	87.04	3.70	3.70	3.70	0	35.19	46.30
2	18.52	78.70	12.96	96.30	2.78	80.56	49.07	64.81	53.70
3	–	21.30	–	–	83.33	15.74	50.93	–	–
4	–	–	–	–	10.19	–	–	–	–

Table 7. Relative allele frequencies (%) of the nine scored enzyme loci of the *Xerocomus chrysenteron* population from Heppenheim.

Allele number	Allele frequencies (%) at locus								
	Acp–B	*Dia–B*	*Dia–C*	*G6pdh–A*	*Lap–B*	*Mdh–C*	*Me–B*	*Pep1–B*	*Pep2–B*
1	45.90	1.64	84.43	5.74	3.28	3.28	6.56	29.51	43.44
2	54.10	85.25	15.57	94.26	2.46	81.97	35.25	70.49	56.56
3	–	13.11	–	–	88.52	14.75	58.20	–	–
4	–	–	–	–	5.74	–	–	–	–

Table 8. Chi-squared (χ^2) values, probability values (P) allocated to the χ^2 values and significance levels (S) at which the number of genotypes deviate from a Hardy-Weinberg distribution in the *X. chrysenteron* population from Bingen and from Heppenheim.

	Acp-B		*Dia-B*		*Dia-C*	
	Bingen	Heppenheim	Bingen	Heppenheim	Bingen	Heppenheim
χ^2	16.3281	25.5127	35.3737	3.3766	17.2363	37.8441
P	$5.33*10^{-5}$	$4.39*10^{-7}$	$1.01*10^{-7}$	0.3371	$3.3*10^{-5}$	$7.66*10^{-10}$
S	***	***	***		***	***
	G6pdh-A		*Lap-B*		*Mdh-B*	
	Bingen	Heppenheim	Bingen	Heppenheim	Bingen	Heppenheim
χ^2	3.1075	0.0403	4.69	0.19	15.24	20.99
P	0.0779	0.8408	0.5845	0.9999	0.00162	0.000106
S					***	***
	Me-B		*Pep1-B*		*Pep2-B*	
	Bingen	Heppenheim	Bingen	Heppenheim	Bingen	Heppenheim
χ^2	10.67	14.93	1.23	0.15	2.5497	4.1667
P	0.013626	0.00188	0.2676	0.7035	0.1103	0.0412
S	*	**				*

Table 9. Genetic identity and genetic distances of the two *Xerocomus chrysenteron* populations Bingen and Heppenheim.

Enzyme locus	Genetic identity *I*	Genetic distance *D*	Genetic distance d_o
Acp–B	0.7999	0.2233	0.3558
Dia–B	0.9936	0.0064	0.0819
Dia–C	0.9994	0.0006	0.0261
G6pdh–A	0.997	0.0003	0.0204
Lap–B	0.9983	0.0017	0.0519
Mdh–B	0.9999	0.0001	0.0141
Me–B	0.9709	0.0295	0.1383
Pep1–B	0.9949	0.0051	0.0568
Pep2–B	0.9984	0.0016	0.0286
Total	0.9782	0.0298	0.0860

Table 10. Heterozygosities (H_L and H_E) of the nine enzyme loci investigated in the *Xerocomus chrysenteron* populations from Bingen and from Heppen-heim.

Enzyme locus	H_L / Bingen	H_L / Heppenheim
Acp–B	0.3018	0.4966
Dia–B	0.3353	0.2558
Dia–C	0.2256	0.2629
G6pdh–A	0.0713	0.1082
Lap–B	0.2931	0.2114
Mdh–B	0.3249	0.3053
Me–B	0.4998	0.5327
Pep1–B	0.4561	0.4160
Pep2–B	0.4973	0.4914
H_e	0.3423	0.3339

Heterozygosity

The average heterozygosity over all investigated gene loci, H_E, was similar in both investigated *X. chrysenteron* populations (Bingen: H_E = 34.23 %; Heppenheim: H_E = 33.39 %) (Table 10) which means that their genetic diversity is very similar. The amount of heterozygosity per locus, however, varied in both populations considerably. The lowest heterozygosity was observed at locus *G6pdh–A* (Bingen: H_L = 7.13 %, Heppenheim: H_L = 19.82 %) (Table 10), while the largest heterozygosity was found at locus *Me–B* (Bingen: H_L = 49.98 %, Heppenheim: H_L = 53.27 %) (Table 10).

Genetic diversity

The total amount of genetic diversity, H_T (NEI 1972) between both *X. chrysenteron* populations was found to be H_T = 34.66 (Table 11). The within population

genetic diversity, H_S, was calculated to be $H_S = 33.81$ % while the genetic diversity between both populations, D_{ST}, was estimated to be $D_{ST} = 0.85$ % (Table 11). The per cent amount of D_{ST} of the total genetic diversity (H_T), G_{ST}, was estimated to be 2.45 % (Table 11). At locus *Mdh–B* the total genetic diversity was found to be lowest within the populations ($D_{ST} = 0$) while the genetic diversity between the two populations was largest at locus *Acp–B* ($G_{ST} = 13.69$ %) (Table 11).

Table 11. Genetic diversities H_S, H_T, D_{ST} and G_{ST}.

Enzyme locus	H_S	H_T	D_{ST}	G_{ST}
Acp–B	0.3992	0.4625	0.0633	0.1369
Dia–B	0.2956	0.2983	0.0027	0.0091
Dia–C	0.2443	0.2446	0.0003	0.0012
G6pdh–A	0.0898	0.0899	0.0001	0.0011
Lap–B	0.2523	0.2534	0.0011	0.0043
Mdh–B	0.3151	0.3151	0	0
Me–B	0.5163	0.5234	0.0071	0.0136
Pep1–B	0.4361	0.4377	0.0016	0.0037
Pep2–B	0.4944	0.4947	0.0003	0.0006
Mean value	**0.3381**	**0.3466**	**0.0085**	**0.0245**

Fixation Index

The fixation index (WRIGHT 1978) was negative in both populations at the loci *Me–B* ($F_{IS\text{-}total} = -0.3762$) and *Pep2–B* ($F_{IS\text{-}total} = -0.2835$) (Table12) which indicates an excess of heterozygous individuals. On the other hand, both populations differ with respect to the number of heterozygous individuals. In the Bingen population, the number is lower than in the Heppenheim population at the loci *G6pdh–A* , *Lap–B* and *Pep1–B* (Table 12).

DISCUSSION

Isozyme banding patterns were interpreted as if the mycelia of *X. chrysenteron* were diploid because they grow in mycorrhizas with beech as a dikaryotic mycelium (BRAND 1989). However, the loci and alleles identified in the fungus are putative. Crosses of monokaryotic isolates are currently under study. It is also assumed that individual samples from the very same tree rootlet were small enough to contain only one fungal individual. DNA analyses of the IGS1 region of the rDNA gene cluster do not contradict to this view (HAESE & ROTHE, in preparation).

Both partners of the symbiotic entity *F. sylvatica*/*X. chrysenteron* show common and differing genetic properties. Both possess, for example, the following common characteristics: the average number of alleles per polymorphic locus (A/L) is relatively high (*X. chrysenteron*: A/L = 2.56; *F. sylvatica*: A/L = 2.3, MÜLLER-STARCK 1991). The genetic variability of both species is much higher within populations than between populations (*X. chrysenteron*: average total genetic distance, $d_{o\text{-}total} = 8.6$ %; *F. sylvatica*: $d_{o\text{-}total} = 5.3$ % (SANDER *et al.*, submitted).

Table 12. Fixation index (F_{IS}) of the nine investigated enzyme loci of the *Xerocomus chrysenteron* populations from Bingen and from Heppenheim as well as the total fixation index of both populations (F_{IS}).

Enzyme locus	F_{IS}		
	Bingen	Heppenheim	Total
Acp–B	0.6318	0.6699	0.7028
Dia–B	0.7238	0.2311	0.5004
Dia–C	0.6717	0.6883	0.6816
G6pdh–A	1.0000	–0.0609	0.3317
Lap–B	0.3681	–0.0858	0.1682
Mdh–B	0.4871	0.4630	0.4748
Me–B	–0.4449	–0.3538	–0.3762
Pep1–B	0.1880	–0.1034	0.0437
Pep2–B	–0.2662	–0.3011	–0.2835

The genetic diversity (NEI 1973) of both symbiotic partners is similar with respect to total genetic diversity (H_T), average genetic diversity within (H_S) and between populations (D_{ST}), and percent amount of D_{ST} on H_T (G_{ST}) (*X. chrysenteron*: $H_T = 0.347$, $H_S = 0.338$, $D_{ST} = 0.009$, $G_{ST} = 0.025$; *F. sylvatica*: $H_T = 0.296$, $H_S = 0.293$, $D_{ST} = 0.003$, $G_{ST} = 0.009$, SANDER *et al.*, submitted).

The average expected heterozygosity varies little between populations of both symbiotic partners (*X. chrysenteron*: 0.338; *F. sylvatica* $H_E = 0.396$, SANDER *et al.*, submitted). On the other hand, the heterozygosity of individual loci varies considerably (*X. chrysenteron*: $H_L = 0.10888$ to 0.533; *F. sylvatica*: $H_L = 0.123$ to 0.6798, SANDER *et al.*, submitted).

Differences between host and symbiotic fungus were observed with respect to the occurrence of alleles and genotypes in their respective populations. Whereas no population specific alleles were found in European beech (BELLETTI & LANTERI 1996; LEONARDI & MENOZZI 1995; KONNERT 1995; LÖCHELT & FRANKE 1995; HATTEMER & ZIEHE 1996) populations specific alleles were observed in *X. chrysenteron* (*Me–B*: allele 1; *Dia–B*: allele 1). The effective number of alleles is lower in the fungus (*X. chrysenteron*: $n_e = 1.55$) than in its host (*F. sylvatica*: $n_e = 2.67$, SANDER *et al.*, submitted). Populations of European beech may show different genotype frequencies at several loci (SANDER *et al.*, submitted) but the two *X. chrysenteron* populations

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).

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