VARIABILITY OF POPULATIONS OF THE ECTOMYCORRHIZAL FUNGUS *XEROCOMUS PRUINATUS* INFECTING EUROPEAN BEECH (*FAGUS SYLVATICA*), SESSILE OAK (*QUERCUS PETRAEA*) OR NORWAY SPRUCE (*PICEA ABIES*)

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Received January 3, 2003; accepted July 14, 2003

ABSTRACT

Allelic frequencies of nine enzyme gene loci were studied in populations of the ectomycorrhizal fungus *Xerocomus pruinatus* (Fr.) Quél. infecting European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.)) or Norway spruce (*Picea abies* (L.) Karst.). The average number of alleles per polymorphic locus was 2.41. In *X. pruinatus* populations from European beech 21 to 23 alleles were observed at the nine loci studied, while 20 alleles were found in a population from sessile oak and 16 alleles occured in a population from Norway spruce. The effective numbers of alleles were n_e = 1.58, 1.72 and 1.80 for the beech, oak and spruce populations, increasing respectively. Also the mean heterozygosities increased from the populations from European beech (H_E = 0.3332) to the one from sessile oak (H_E = 0.4659) to the one from Norway spruce (H_E = 0.5622). Genetic distances were smallest between fungal populations infecting the same host species (beech: d_0 = 0.091), whereas genetic distances were larger for populations infecting different tree species (oak/spruce: d_0 = 0.1717, beech/spruce: d_0 = 0.2219, beech/oak: d_0 = 0.2595).

In individuals on European beech two loci coded for the enzyme system diaphorase, whereas one diaphorase locus was active in mycorrhizae on sessile oak and no diaphorase activity was observed in individuals infecting Norway spruce roots. The host-dependent diaphorase expressions in *X. pruinatus* are discussed as adaptations of this ectomycorrhizal fungus to its host's metabolism.

Key words: ectomycorrhiza, *Fagus sylvatica* (L.), isozymes, *Picea abies* (L.) Karst., population genetics, *Quercus petraea* (Matt.) Liebl., symbiosis, *Xerocomus pruinatus* (Fr.) Quél.

Introduction

Most tree species in European forests form, on their very fine roots, an ectomycorrhizal symbiosis with basidiomycetal and ascomycetal fungi (SMITH & READ 1997). In this mutualistic association, the fungi improve the water and nutrient supply of their hosts, whereas the hosts support the fungi with photosynthetic products (DÄHNE *et al.* 1995), especially carbohydrates (HARLEY & SMITH 1983).

One basidiomycetal fungus which can interact with deciduous and coniferous tree species in European forest ecosystems is *Xerocomus pruinatus* (Fr.) Quél. It forms a silvery-white to light yellow ectomycorrhiza with root tips of e.g. European beech, oak and Norway spruce.

The genetic variability of European beech (MÜLLER-STARCK & STARKE 1993, KONNERT 1995, LENONARDI & MENOZZI 1995, LÖCHELT & FRANKE 1995, BELLETI & LANTERI 1996, HATTEMER & ZIEHE 1996, SANDER *et al.* 2000, 2001), oak (MÜLLER-STARCK & ZIEHE 1991, LÖCHELT 1993, STREIFF *et al.* 1998) and Norway spruce (MORGANTE & VENDRAMIN 1991, GEBUREK 1999) have been studied intensely, while studies on the genetic variabilities of their ectomycorrhizal fungi are rare (MELTZER & ROTHE 2000).

Fruiting bodies (basidiocarps) may be taken to study the genetic variability of their populations as has been done with symbionts on conifers such as *Hebeloma cylindrosporum* (GRYTA *et al.* 1997, GUIDOT *et al.* 1999), *Laccaria bicolor* (BASTIDE *et al.* 1995) and *Suillus* sp. (ZHU *et al.* 1988, SEN 1990, KELLER 1992, EL KARKOURI *et al.* 1996, BONELLO *et al.* 1998), however, fruiting bodies may not represent the full below ground variability. Moreover, in these studies mostly isolates of fruiting bodies were used (ZHU *et al.* 1988, KELLER 1992). In these tissues isozyme patterns may be different from the original tissue so that genetic patterns may be misinterpreted. Because of this reason we studied exclusively the mycorrhizae of forest trees.

Ectomycorrhizal fungi which can infect more than one tree species face the problem of adapting to the different metabolisms of their hosts and their habitats. In the present study we considered both problems by analyzing the population structure of *X. pruinatus* on European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* Liebl.) and Norway spruce (*Picea abies* (L.) Karst.) in central Germany.

MATERIAL AND METHODS

Stand characteristics

Fine roots ($d \leq 2$ mm) which were infected at their tips with *Xerocomus pruinatus* (Fr.) Quél. were sampled in a beech (*Fagus sylvatica* L.) stand, a sessile oak (*Quercus petraea* Liebl.) stand, and a Norway spruce (*Picea abies* (L.) Karst.) stand in central Germany. At least 47 trees per stand were probed. The stand characteristics are given in Table 1, an overview of their localization is shown in Fig. 1.

Root sampling

In the beech forest site in Rüdesheim mycorrhizae of *X. pruinatus* infecting *Fagus sylvatica* were taken from the end of April to the end of May, 1999. In the sessile oak woods (Trippstadt population) mycorrhizae of *X. pruinatus* associated with *Quercus petraea* were sampled between mid-September and the end of October, 1999. In the Norway spruce forest (Taunusstein population) mycorrhizae of *X. pruinatus* on *Picea abies*

Table 1. Characteristics of the investigated European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* Liebl.) and Norway spruce (*Picea abies* (L.) Karst.) stands in central Germany.

Stand characteristics	Rüdesheim	Trippstadt	Taunusstein
Forest district	Weissenturm; Stadtwald Geisenheim	Leimen	Altenstein; Stadtwald Chausseehaus
Forest site	Rüdesheim	Merzalben	Chausseehaus
Stand number	63	I, 10 a	165 c
Stand area (ha)	9.8	11	3.6
Partition of tree species	beech: 87 %, oak: 6 %, hornbeam: 5 %	sessile oak: 100 % (understory: beech)	Norway spruce: 100 %
Altitude (m above sealevel)	265–380	550	465–501
Mean temperature (°C) per year (per vegetation period)	7.5 (14)	7.5–8.0 (13.7)	7.5 (13)
Mean precipitation (mm) per year (per vegetation period)	612 (294)	950 (410)	800 (200)
Parent rock	Shale with loess loam	mittlerer Buntsandstein (new red sandstone)	quartzite with loess loam
Liming (year)	yes (1995)	no	yes (1990)
Application rate (t/ha)	3		3
Host species	*F. sylvatica*	*Q. petraea*	*P. abies*
Age of trees	123–126	98	78
Number of mycorrhizae sampled	56	54	47
Date of sampling of mycorrhizae	spring 1999	autumn 1999	spring 2000
Population code	Rüd	Tri	Tau

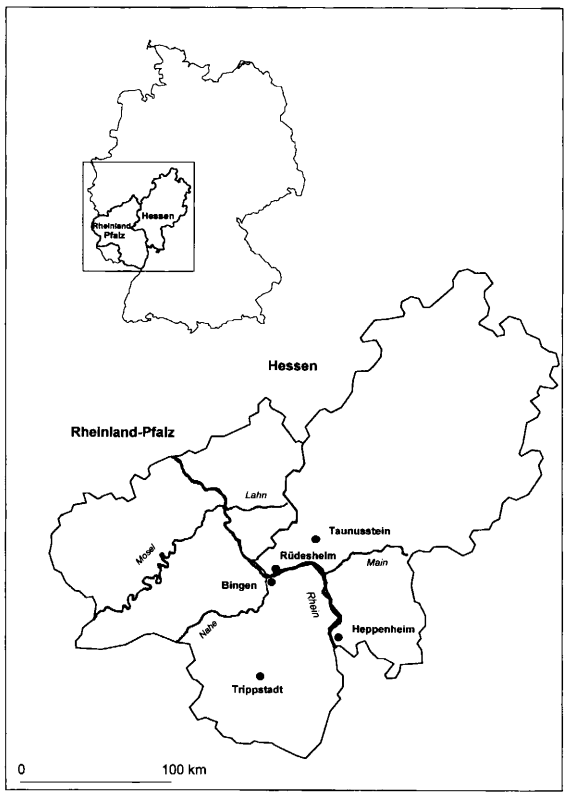

Figure 1. Location of the states Hesse and Rhineland-Palatinate in central Germany (upper picture) and location of forest sites within these states (lower picture) where *Xerocomus pruinatus* populations were sampled. At the forest sites Bingen, Heppenheim (MELTZER & ROTHE 2000) and Rüdesheim the fungus infected *Fagus sylvatica*, at Trippstadt it was associated with *Quercus petraea* and at Taunusstein it formed mycorrhizae with *Picea abies*.

were dug out from end of April to end of of May, 2000. Sampling and cleaning of mycorrhizae was performed as described recently (MELTZER & ROTHE 2000).

The mycorrhizae were identified initially under a stereomicroscope at a 25-fold magnification, according to the morphological criteria described by BRAND (1989) and AGERER (1988–98), and later by DNA-analysis (HAESE & ROTHE 2003). The remaining mycorrhizal clusters on the same root were then carefully separated, isolating the root tips infected with dikaryotic *X. pruinatus* and non-infected proximal parts (d = 1 to 2 mm) (MELTZER & ROTHE 2000). Mycorrhizal root systems having a fresh weight of at least 50 mg were put into a 1.5 ml Eppedorf-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 in a similar fashion.

Identification of *Xerocomus pruinatus*

The silvery-white mycorrhiza was initially identified according to the morphological descriptions reported by BRAND (1989) as *Xerocomus chrysenteron*. PCR-RFLP analysis of the ITS region of the rDNA gene cluster showed that all of the investigated individuals were from the same species. During the course of the research however, it was found that the ectomycorrhizal species described by BRAND (1989) was possibly not *Xerocomus chrysenteron* but rather *Xerocomus pruinatus* (AGERER 2002, personal communication).

Subsequently, the species identification using PCR-RFLP analysis was re-examined, using fruiting bodies obtained from Hahn and Agerer's herbarium for comparison (*X. pruinatus* (leg./det. Ch. Hahn, 27.10. 1999, CH 305/99). PCR-RFLP analysis of the ITS showed beyond a doubt that the silvery-white mycorrhizae used in our study were identical to *X. pruinatus* and not *X. chrysenteron* (HAESE & ROTHE 2003).

In the literature however, there is scarcely any reference at all of *Xerocomus pruinatus* which is described as a rare or seldom occuring mushroom (PHILIPPS 1998). This stands in contradiction to our field observations. In deciduous and coniferous forests in middle Germany, the mycorrhizae of *X. pruinatus* were abundant.

Enzyme analysis

Enzymes were extracted from mycorrrhizal and non-mycorrhizal roots, submitted to cellulose-acetate electrophoresis and visualized as described recently (MELTZER & ROTHE 2000).

Exploitation of electrophoretic data

The hyphae of the fungus *X. pruinatus* are dikaryotic. Therefore, electrophoretic enzyme patterns were translated into genotypes and alleles at gene loci according to known enzymatic structures of diploid material (cf. MELTZER & ROTHE 2000). Definition and utilization of mathematical formula were as given by ROTHE (1994) and MELTZER & ROTHE (2000).

RESULTS

Investigated enzyme loci

The polymorphic enzyme loci *Acp-B*, *Dia-B*, *Dia-C*, *G6pdh-A*, *Lap-B*, *Mdh-C*, *Me-B*, *Pep1-B* and *Pep2-B*

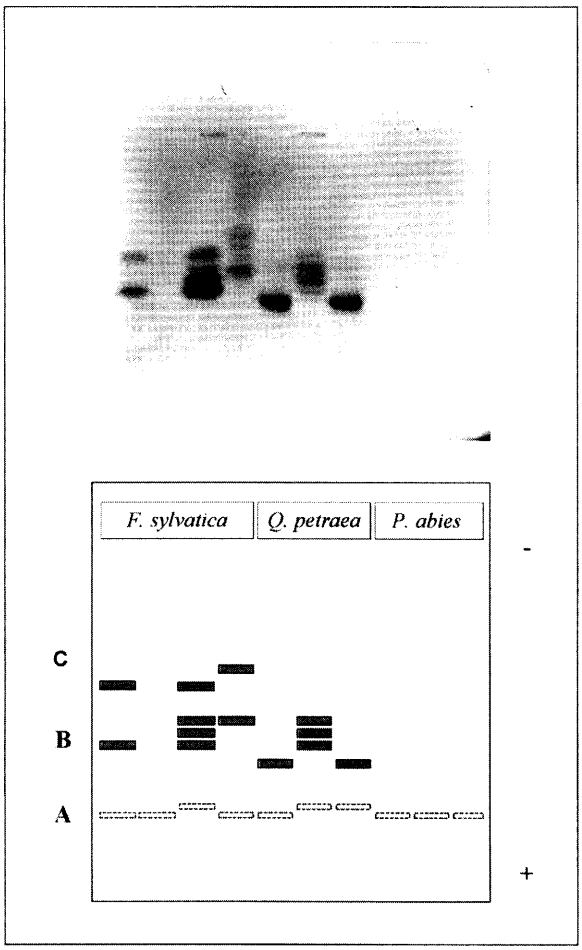

Figure 2. Zymogramm (upper picture) and schematic representation (lower picture) of diaphorase allozymes of mycorrhizae of *Xerocomus pruinatus* associated with *Fagus sylvatica*, *Quercus petraea* or *Picea abies*. The locus *Dia-A* is host-specific, since it occured in non-mycorrhizal roots (line 2 from left). The loci *Dia-B* and *Dia-C* were both active in mycorrhizae on European beech (lines 1, 3 and 4 from left to right), in association with sessile oak the locus *Dia-B* was exclusively active (lines 5 to 7) while both loci *Dia-C* and *Dia-B* were inactive in mycorrhizae on Norway spruce (lines 8 to 10). Genotypes from left to right: *Dia-C*: 1/1, control , 1/1 and 2/2 (Rüdesheim population), *Dia-B*: 2/2, control, 2/3, 3/3 (Rüdesheim population) and 1/1, 2/3, 1/1 (Trippstadt population). Control: Extract of non-mycorrhizal fine roots (1–2 mm in diameter).

served as genetic markers. These loci were fungus-specific irrespective of the three host species (MELTZER & ROTHE 2000). On the other hand, it resulted that in association with European beech all of the nine loci coded for active enzymes while in symbiosis with sessile oak, one locus, *Dia-C* was inactive, and in symbiosis with Norway spruce two loci, *Dia-B* and *Dia-C* were inactive (Fig. 2).

Polymorphic loci and effective number of alleles

In a previous study (MELTZER & ROTHE 2000) the same nine enzyme loci which were studied here, were investigated in two *Xerocomus pruinatus* populations on European beech. In these populations the number of alleles at the nine loci was similar to the number observed in the population on European beech studied here, where a total number of 22 alleles was found. The number of alleles observed in the fungal population on oak was 20 and the one observed in *X. pruinatus* on Norway spruce was 16. The decreasing number of alleles results from the missing activity of one *Dia*-locus in association with sessile oak and the missing activity of two *Dia*-loci in symbiosis with Norway spruce.

The average numbers of alleles per polymorphic locus (*a/l*) were 2.33, 2.55 and 2.44 when the fungus was associated with beech (populations Bingen, Heppenheim and Rüdesheim, respectively), while *a/l* was = 2.50 in association with sessile oak and 2.29 when the fungus infected Norway spruce (Taunusstein population).

In accordance with the total number of alleles at the nine loci, the effective number of alleles also depended on the host with which *X. pruinatus* formed an ectomycorrhizal association. The effective number of alleles n_e was largest in association with spruce, then came oak, then beech (Table 3).

Genotype frequencies

Including the two *X. pruinatus* populations on beech which we studied recently (MELTZER & ROTHE 2000) a total of 38 genotypes were found at the nine polymorphic loci investigated. The average number of genotypes per polymorphic locus (*g/l*) was 3.41 for the populations on European beech, 3.0 in association with oak and 2.78 when Norway spruce was infected (Table 2). Thus, the number of genotypes was higher in the populations on deciduous trees and lower in the population on the coniferous species.

In three out of the five *X. pruinatus* populations studied so far, each had at least one genotype which appeared to be population specific (Heppenheim: *Me-B* 1/1 and *Me-B* 1/2, Rüdesheim: *Mdh-C* 1/3 and Trippstadt: *Dia-B* 1/2). This specificity seems to be uninfluenced by the host.

Table 2. Genotype frequencies (%) and standard deviations in *X. pruinatus* populations from European beech, sessile oak or Norway spruce.

Locus	Genotype	Rüdesheim	Trippstadt	Taunusstein
		beech	oak	spruce
Acp-B	1/1	64.29±12.55	44.44*±13.25	36.17±13.74
	1/2	17.86±10.03	11.11±8.38	21.28±11.70
	2/2	17.86±10.03	44.44±13.25	42.55±14.14
Dia-B	1/1	0*	55.56*±13.25	0*
	1/2	0	11.11*±8.38	0
	2/2	82.14*±10.03	18.52*±10.36	0*
	2/3	14.29±9.17	14.81±9.47	0*
	3/3	3.57±4.86	0	0
Dia-C	1/1	91.07*±7.47	0*	0*
	1/2	5.36±5.90	0	0
	2/2	3.57±4.86	0	0
G6pdh-A	1/1	1.79±3.47	12.96±8.96	21.28±11.70
	1/2	0*	27.78*±11.95	10.64±8.82
	2/2	98.21*±3.47	59.26*±13.11	68.09±13.33
Lap-B	1/1	0	0	6.38±6.99
	1/3	0*	18.52±10.36	12.77±9.54
	2/3	3.57±4.86	5.56±6.11	0
	3/3	82.14±10.03	74.07±11.69	72.34±12.79
	3/4	10.71±8.10	1.85±3.59	4.26±5.77
	4/4	3.57±4.86	0	4.26±5.77
Mdh-C	1/1	12.5±8.66	20.37±10.74	10.64±8.82
	1/2	0	11.11±8.38	2.13±4.13
	1/3	1.79±3.47	0	0
	2/2	57.14±12.96	64.81±12.74	59.57±14.03
	2/3	17.86±10.03	3.70±5.03	8.51±7.98
	3/3	10.71±8.01	0*	19.15±11.25
Me-B	1/1	0	0	0
	1/2	0	0	0
	1/3	5.36±5.90	0	0
	2/2	23.21±11.06	77.78*±11.09	17.02±10.74
	2/3	60.71±12.79	20.37*±10.74	51.06±14.29
	3/3	10.71±8.01	1.85*±3.59	31.92±13.33
Pep1-B	1/1	16.07±9.62	7.41±6.99	12.77±9.54
	1/2	41.07±12.89	46.30±13.30	44.68±14.21
	2/2	42.86±12.96	46.30±13.30	42.55±14.14
Pep2-B	1/1	30.36±12.04	14.81±8.96	4.26±5.77
	1/2	51.79±13.09	64.81±11.95	44.68±14.21
	2/2	17.86±10.03	20.37±13.11	51.06*±14.29
Average number of genotypes per locus (*g/l*)		3.33	3.00	2.78

X. pruinatus infected at stand Rüdesheim *Fagus sylvatica*, at stand Trippstadt *Quercus petraea* and at stand Taunusstein *Picea abies*. The asterix (*) indicates a significant difference ($\alpha = 5$ %) in frequency between the respective population and the group of the four remaining populations which are composed of the two beech populations listed in the table and the two beech populations investigated recently (Bingen and Heppenheim; MELTZER & ROTHE 2000).

Table 3. Allele frequencies (%), standard deviations and effective number of alleles (n_e) of the nine scored enzyme loci in *X. pruinatus* populations infecting European beech, sessile oak or Norway spruce.

Locus	Allele number	Rüdesheim	Trippstadt	Taunusstein
		beech	oak	spruce
Acp-B	1	73.21*±0.0418	50.00*±0.0481	46.81*±0.0515
	2	26.79*±0.0418	50.00*±0.0481	53.19*±0.0515
	n_e	1.65	2.00	1.99
Dia-B	1	0*	61.11*±0.0469	0*
	2	89.29*±0.0292	31.48*±0.0447	0*
	3	10.71±0.0292	7.41±0.0252	0*
	n_e	1.24	2.09	-
Dia-C	1	93.75*±0.0229	0*	0*
	2	6.25±0.0229	0*	0*
	n_e	1.13	-	-
G6pdh-A	1	1.79*±0.0125	26.85*±0.0426	26.60*±0.0456
	2	98.21*±0.0125	73.15*±0.0426	73.40*±0.0456
	n_e	1.04	1.65	1.64
Lap-B	1	0*	9.26±0.0279	12.77*±0.0344
	2	1.79*±0.0125	2.78±0.0158	0*
	3	89.29±0.0292	87.04±0.0323	80.85±0.0456
	4	8.93±0.0269	0.93*±0.0092	6.38±0.0252
	n_e	1.24	1.30	1.48
Mdh-C	1	13.39±0.0322	25.93*±0.0422	11.70±0.0332
	2	66.07*±0.0447	72.22±0.0431	64.89*±0.0492
	3	20.54±0.0382	1.85*±0.013	2.34*±0.0437
	n_e	2.01	1.70	2.04
Me-B	1	2.68±0.0153	0*	0*
	2	53.57±0.0292	87.96*±0.0313	42.55*±0.051
	3	43.75±0.0269	12.04*±0.0313	57.45*±0.051
	n_e	2.09	1.27	1.96
Pep1-B	1	36.61±0.0455	30.56±0.0443	35.11±0.0492
	2	63.39±0.0455	69.44±0.0443	64.89±0.0492
	n_e	1.87	1.74	1.84
Pep2-B	1	56.25*±0.0469	47.22±0.048	26.60*±0.0456
	2	43.75*±0.0469	52.78±0.048	73.40*±0.0456
	n_e	1.97	1.99	1.64
	Mean value n_e	1.58	1.72	1.80

X. pruinatus infected at stand Rüdesheim *Fagus sylvatica*, at stand Trippstadt *Quercus petraea* and at stand Taunusstein *Picea abies*. The asterix (*) indicates a significant difference in frequency between the respective population and the group of four remaining populations which are composed of the two beech populations listed in the table and the two beech populations investigated recently (Bingen and Heppenheim; MELTZER & ROTHE 2000).

On the other hand, some host specific genotype frequencies seem to exist: for example genotype *Dia-B* 1/1 was most frequent in individuals on oak (55.56 %) while it was extremely rare in populations on beech (0 to 1.64 % (MELTZER & ROTHE 2000)), and was missing in the population on Norway spruce. The genotype *Dia-B* 2/2 was most frequent in populations on beech (77.21 % (MELTZER & ROTHE 2000) while it occured in much lower frequencies in oak (18.52 %) and it was missing in association with Norway spruce. The genotype *Me-B* 2/2 was most frequent in association with sessile oak (77.78 %, Table 2) while its frequencies were much lower in association with beech (3.28 to 23.21 %) and Norway spruce (17.02 %) (Table 2).

Allele frequencies

In the population which infected sessile oak (Trippstadt), the two alleles at locus *Dia-C* were missing, which were present when the fungus infected European beech (Table 3). Also allele $Me\text{-}B_1$ was not found in the fungal population on oak. But this is not a host specific phenomenon since this allele was also missing in one population on beech (Bingen; MELTZER & ROTHE 2000). The absence of allele $Lap\text{-}B_2$ in association with Norway spruce is also most probably not a host specific effect since the allele is rare in the remaining four populations (Table 3). In addition the absence of allele $Me\text{-}B_1$ when infecting Norway spruce cannot be attributed to the host species, since the allele can also be absent in association with European beech (Bingen; MELTZER & ROTHE 2000) and sessile oak (Trippstadt) (Table 3).

Some other alleles seem to appear in host specific frequencies such as $Dia\text{-}B_1$, $Mdh\text{-}C_1$ and $Me\text{-}B_2$ which were much more frequent in association with sessile oak than with beech and Norway spruce. $Mdh\text{-}C_3$ was more frequent when beech was infected than in association with oak and spruce (Table 3). $Pep2\text{-}B_2$ was much more dominant in the population from Norway spruce than in the remaining populations (cf. MELTZER & ROTHE 2000, Table 3). In symbiosis with oak the two alleles at locus *Dia-C* were completely missing while in association with Norway spruce the alleles at locus *Dia-B* and *Dia-C* were not expressed.

Significant differences between the population Bingen and the remaining four populations were found for 12 alleles, significant differences between the Heppenheim population and the remaining four populations were observed for 14 alleles.

Genetic distances d_0 between populations

The genetic distances between the 10 possible pairs of populations investigated so far ranged at the different loci from d_0 = 0.0091 (locus *Acp-B* (Heppenheim/Taunusstein)) to d_0 = 0.6111 (locus *Dia-B* (Bingen/Trippstadt, Rüdesheim/Trippstadt)).

Genetic distances were lowest between *X. pruinatus* populations which infected trees of the same species (European beech: Bin/Rüd: d_0 = 0.0739, Bin/Hep: d_0 = 0.0860, Hep/Rüd: d_0 = 0,1141; mean: d_0 = 0.091) but were larger between populations which infected different tree species (Fig. 3). The genetic distance between the *X. pruinatus* population on Norway spruce (Taunusstein) and the one on sessile oak (Trippstadt) was d_0 = 0.1717 (Fig. 3). An even larger difference was calculated for the populations on European beech (Bingen, Heppenheim, Rüdesheim) and the one infecting Norway spruce (Taunusstein), where the average genetic distance was d_0 = 0.2219 (Fig. 3). The largest genetic distance was observed between the populations infecting European beech and the one on sessile oak (Trippstadt) where the average value was d_0 = 0.2595 (Fig. 3). As a result the largest distances were found for the populations on beech and the one on sessile oak.

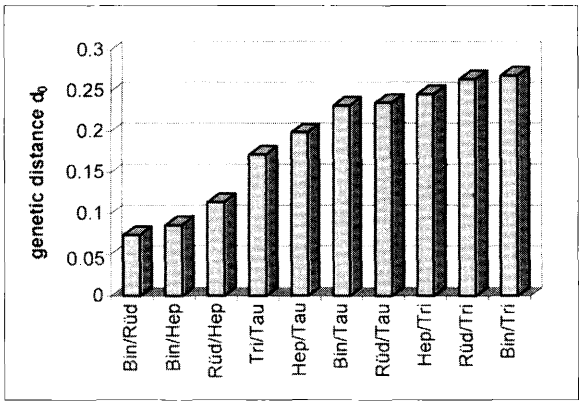

Figure 3. Genetic distances d_0 between the 10 pairs of populations of *Xerocomus pruinatus*, including the two on beech (Bingen, Heppenheim) which were investigated before (MELTZER & ROTHE 2000). The *X. pruinatus* populations investigated here infected either European beech (Rüdesheim), sessile oak (Trippstadt) or Norway spruce (Taunusstein).

Subpopulation differentiation

Genetic distances between each of the investigated *X. pruinatus* populations and the remaining four populations (including the two investigated recently (MELTZER & ROTHE 2000)) are comparable to the pairwise genetic distances. Related to the five populations investigated so far, the average gene pool value was δ = 0.1630

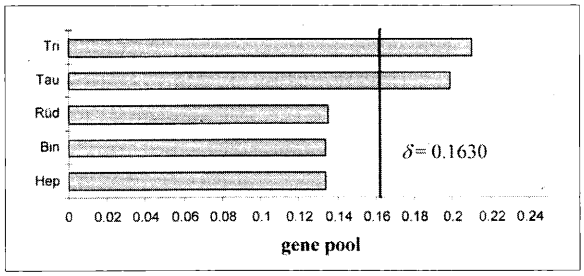

Figure 4. Subpopulation differentiation D_j between one of the five *Xerocomus pruinatus* populations including the two investigated before (Bingen, Heppenheim; MELTZER & ROTHE 2000) and the remaining complement of the four populations. The populations which infected European beech were sampled at the forest sites Bingen, Heppenheim and Rüdesheim, the population infecting sessile oak was collected at the Trippstadt site, while representatives of the population on Norway spruce came from Taunusstein. δ= average gene pool value.

(Fig. 4). The gene pool values between *X. pruinatus* on European beech and the remaining complement were the lowest (mean gene pool = 0.1343), while those between the fungal population on sessile oak and the remaining complement were highest (gene pool = 0.2113; Fig. 4).

Genetic diversity

The total amount of genetic diversity, H_T (Nei 1973), between the five investigated *X. pruinatus* populations was found to be H_T = 0.4673. The intra-population genetic diversity, H_S, was calculated to be H_S = 0.4056 while the genetic diversity between the five populations, D_{ST}, was estimated to be D_{ST} = 0.0617. The per cent amount of D_{ST} on the total genetic diversity (H_T), G_{ST}, was estimated to be 0.1096. The lowest genetic diversity between populations at one of the investigated loci was found at locus *G6pdh-A* (H_T = 0.2253) while the largest diversity was found at locus *Dia-C* (H_T = 0.7138) which was exclusively active when *X. pruinatus* was infecting European beech.

Mean heterozygosity

Within the five investigated *X. pruinatus* populations, the average heterozygosity at one of the nine loci ranged from 0.0352 (*G6pdh-A*: Rüdesheim population) to 1.000 in those populations where the loci *Dia-B* (Norway spruce, Taunusstein population) and *Dia-C* (Norway spruce, Taunusstein population and sessile oak, Trippstadt population) were inactive (Table 4). The average heterozygosity over all loci and populations was H_S = 0.4055. It ranged from H_S = 0.1996 at locus *G6pdh-A* to H_S = 0.5211 at locus *Dia-C* (Table 4). Related to the five populations investigated so far, the average heterozygosity over all investigated loci was lowest in the three *X. pruinatus* populations on European beech (H_E = 0.3332) as compared to the population on sessile oak (H_E = 0.4659) and Norway spruce (H_E = 0.5622; Table 4).

DISCUSSION

In ectomycorrhizae of *X. pruinatus,* nine enzyme loci could be attributed to the fungus. Among these were two loci which code for enzymes with diaphorase activity when the fungus infected European beech. In the population which infected sessile oak only one of these two diaphorase loci was active. Neither of the two diaphorase loci which exist in *X. pruinatus* were expressed in populations on Norway spruce. This

Table 4. Heterozygosities H_I, H_E and H_S of the nine enzyme loci investigated in the *X. pruinatus* populations, including the two populations previously studied (Bingen and Heppenheim; MELTZER & ROTHE 2000).

Locus	H_I/Bin	H_I/Hep	H_I/Rüd	H_I/Tri	H_I/Tau	H_S
Acp-B	0.3018	0.4966	0.3923	0.5000	0.4980	0.4377
Dia-B	0.3353	0.2558	0.1913	0.5220	1.0000	0.4609
Dia-C	0.2256	0.2629	0.1172	1.0000	1.0000	0.5211
G6pdh-A	0.0713	0.1082	0.0352	0.3928	0.3905	0.1996
Lap-B	0.2931	0.2114	0.1944	0.2330	0.3260	0.2516
Mdh-C	0.3249	0.3053	0.5034	0.4108	0.5105	0.4110
Me-B	0.4998	0.5327	0.5209	0.2118	0.4889	0.4508
Pep1-B	0.4561	0.4160	0.4641	0.4244	0.4557	0.4433
Pep2-B	0.4973	0.4914	0.4922	0.4985	0.3905	0.4740
H_E	0.3339	0.3423	0.3234	0.4659	0.5622	0.4055

observation indicates host-specific adaptation of the fungus to metabolic differences in the respective tree species resulting in an energy sharing system which enhances symbiotic inter-dependency and specificity.

Diaphorase (dihydrolipoamide reductase (NAD^+), lipoyl dehydrogenase, lipoamide dehydrogenase (NADH), lipoamide reductase (NADH), EC 1.6.4.3 (Enzyme nomenclature 1979)) is a flavoprotein and a component of the multienzyme pyruvate dehydrogenase complex and the 2-oxoglutarate (a-ketoglutarate) dehydrogenase complex (Enzyme nomenclature 1979). The pyruvate dehydrogenase complex occurs in plants in plastids and mitochondria; the a-ketoglutarate dehydrogenase complex is part of the mitochondrial citrate cycle (STREYER 1996).

In leaves of European beech two diaphorase loci have been identified, locus *Dia-A* with two alleles coding for monomeric enzymes, and locus *Dia-B* comprising three alleles coding for tetrameric enzymes (SANDER *et al.* 2000, 2001). In non-mycorrhizal roots of European beech only one of these two loci is active. Besides this locus, two *Dia*-loci could be exclusively attributed to the hyphae of *X. pruinatus*. They both may originate from the mitochondria of the fungus. If so, one of the diaphorase loci could code for enzymes which are part of the pyruvate dehydrogenase complex while the second locus could code for diaphorases of the a-ketoglutarate dehydrogenase complex. Thus, in association with beech, the fungus would have a complete citric acid cycle working, which may correspond to a higher energy demand (cf. MÜLLER & LOEFFLER 1992).

In mycorhizae of *X. pruinatus/Picea abies* no fungus-specific diaphorase enzymes could be visualized. Possibly, this indicates that here neither the fungal pyruvate dehydrogenase-complex nor the fungal a-ketoglutarate dehydrogenase-complex is active. If this is so, then the fungus probably uses the glyoxylic acid cycle instead of the citric acid cycle. Acetate and glycine seem to induce the glyoxylic acid cycle in fungi (MÜLLER & LOEFFLER 1992). The glyoxylic acid cycle is used under the conditions of high ATP-levels. Thus in association with Norway spruce roots the mycelia of *X. pruinatus* could be well supplied with energy whereas in association with roots of European beech they may lack of sufficient energy. In mycorrhizal roots of Norway spruce we found citric acid, malic acid and oxalic acid to predominate (SCHWANZ *et al.* 1993).

It might be that in association with sessile oak *X. pruinatus* also uses the citric acid cycle to gain its energy but then hydrogen would not be imported into the mitochondria via pyruvate but via organic acids since only one of the diaphorase loci is active and its enzymes might be involved in the a-ketoglutarate dehydrogenase complex.

The allelic variability of *X. pruinatus* populations on beech and oak was a little higher (*a/l* = 2.44 and 2.22) as compared to the population infecting Norway spruce (*a/l* = 1.78) and this seems to be true for the corresponding host populations as well (beech: *a/l* = 2.68 (SANDER *et al.* 2000, MÜLLER-STARCK & ZIEHE 1991), oak: *a/l* = 2.7 (LÖCHELT 1993, MÜLLER-STARCK & ZIEHE 1991) and spruce *a/l* = 2.0 (GEBUREK 1999)). This would mean that the fungus adapted its genetic variability to the genetic variability of its respective hosts. This process may have involved shared and differential protein synthesis. With more various substrates provided by the host a subsequent loss of functional variability within the fungal symbiont may occur over time. Vice versa, with less variable substrates, the fungal symbiont would need active complementary systems and increased complexity. This hypothesis is confirmed by the average number of genotypes per polymorphic locus which was somewhat higher in *X. pruinatus* populations infecting beech (*g/l* = 3.41) or oak (*g/l* = 3.0) as compared to Norway spruce (*g/l* = 2.78). In association with deciduous trees, the number of genotypes is probably larger than in association with conifers because during the evolution from conifers to deciduous tree species additional genotypes accumulated.

Contrasting to this situation the mean heterozygosities in *X. pruinatus* populations increased from H_E = 0.3332 in association with European beech to H_E = 0.4656 in association with sessile oak to H_E = 0.5622 in symbiosis with Norway spruce. This points to the need for a certain heterosis effect in association with conifers as compared in association with deciduous tree.

Some genotypes of *X. pruinatus* were host independent in frequency but population specific such as the genotypes *Me-B* 1/1, *Me-B* 1/2 (beech, Heppenheim population) and *Mdh-C* 1/3 (beech, Rüdesheim population), which may imply local hot spots of possible evolutionary change.

The per cent amount of genetic diversity between the five populations (D_{ST}) on the total genetic diversity (H_T) was estimated to be G_{ST} = 11 %. This value equals the one estimated for forest trees where the largest genetic diversity was also found within populations. It follows that the founding strategy of the fungus must resemble the one of its hosts.

ACKNOWLEDGEMENT

This work was supported financially by the Environmental Research Center of the Johannes Gutenberg-University Mainz.

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