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Genetic variation in *Heterobasidion abietinum* populations detected with the M13 minisatellite marker

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Summary

Amplification profiles of the M13 minisatellite core sequence from 47 isolates of *Heterobasidion annosum s.l.*, collected mostly from *Abies alba* in different European countries, were analysed in comparison with profiles of four isolates of *H. annosum s.s.* and three isolates of *H. parviporum*. Genetic variation within and among groups of populations was studied by analysis of molecular variance. A dendrogram constructed with the Neighbor-Joining method differentiated the three species from each other. Isolates of *H. abietinum* from Balkan peninsula, Italian peninsula and French Pyrenees each separated into clusters according to geographical origin. Such clear geographic clustering was not detected among isolates from central Europe. The variations between *H. abietinum* isolates in relation to the migration history of *Abies* are discussed.

1 Introduction

Heterobasidion abietinum Niemelä & Korhonen (the 'F type' of *H. annosum s.l.*) is a root rot fungus with a distribution area predominantly in the south of Europe, with main hosts in the genus *Abies*. The known distribution of the fungus extends to Spain in the west, to southern Poland in the north, to the western Caucasus in the east and to southern Greece and Italy in the south (Woodward et al. 1998; Korhonen and Dai 2005; Sánchez et al. 2005). Root and butt rot caused by *H. abietinum* is economically important in forests of *A. alba* in Italy and of *A. cephalonica* and *A. borisii-regis* in Greece, where *Abies* spp. are under stress from the dry summer climate (Korhonen et al. 1998). In central Europe and the Pyrenees, where summers are more humid, *H. abietinum* is mostly a saprophyte on the stumps and logs of *Abies,* and has only minor economic importance (Munda 1994; Łakomy 1996).

The distribution of *H. abietinum* in Europe is more restricted than that of *H. annosum s.s.* and *H. parviporum*. It is likely that *H. abietinum* is limited to Europe, including neighbouring regions in the Middle East and northern Africa, where the western Eurasian species of *Abies* occur (Dai et al. 2003; Doğmuş-Lehtijärvi et al. 2006).

Hitherto, the different geographical populations of *H. abietinum* were studied only by La Porta et al. (1997), in an investigation dealing with *H. abietinum* in Italy alone. Analysis of randomly amplified polymorphic DNA (RAPD) markers of *H. abietinum* isolates from a number of sites suggested that the isolates tended to group at the level of the larger geographical areas. Isolates from southern Italy showed the greatest genetic variation, while the northern isolates (from the Alpine regions) showed the least.

The present study examined populations of *H. abietinum* from different parts of Europe by analysing profiles obtained following direct amplification of minisatellite DNA-PCR

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(DAMD-PCR), using the minisatellite M13 as a primer. This method was previously applied to studies of *Heterobasidion* by Stenlid et al. (1994), and also to examine the geographical differentiation of other fungal populations such as *Ceratocystis platani*, *Ophiostoma ulmi* and *Nectria fuckeliana* (Vasiliauskas and Stenlid 1997, 1998; Hogberg and Stenlid 1999; Hogberg et al. 1999; Santini and Capretti 2000; Santini et al. 2005). The aim of this work was to determine if the genetic variation of *H. abietinum* isolates sampled in the Mediterranean basin and in central Europe presented a geographical structure. Any such geographical structure may be connected with the migration history of *A. alba*, the main host of *H. abietinum*.

2 Materials and methods

Forty-seven isolates (38 homokaryotic and nine heterokaryotic) were examined: 40 of *H. abietinum*, four of *H. annosum s.s.* and three of *H. parviporum* (Table 1). *H. abietinum* isolates came from different geographical regions: central Europe (Poland, Austria, Switzerland), the Italian peninsula, the Balkan peninsula (Greece, Bulgaria, Slovenia) and French Pyrenees. The four *H. annosum s.s.* isolates were from Austria and the three *H. parviporum* isolates from Austria (one) and Poland (two). These last two species were included in the test for comparison.

2.1 DNA extraction and amplification

Isolates were grown in 2.4% potato dextrose broth (PDB). After 2 weeks of incubation at 23°C mycelium was collected on filter paper by vacuum filtration. DNA was extracted using the procedure of Smith and Stanosz (1995). Amplification profiles were obtained in PCR experiments using the M13 minisatellite core sequence (5′-AGGGTGGCGGTTCT-3′; Innovagen, Lund, Sweden; Karlsson 1993). Amplifications were performed in volumes of 25 μl containing: Tris-HCl 10 mM, pH 8; dNTPS 0.2 mM; $MgCl_2$ 2.5 mM; *Taq* 1 U/μl; minisatellite M13 12.7 μM; 20 ng of fungal DNA. The thermal cycler was programmed for a predenaturation step at 93°C for 3 min, followed by 45 cycles at 93°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min.

About 8 μl of each amplification product was loaded into 1.4% agarose gel with TBE buffer (40 mM Tris-borate, 1 mM EDTA, pH 8) and 0.5 μg/ml ethidium bromide. Electrophoresis was carried out at 90 V for 2.5 h. Gels were visualized through UV fluorescence and photographed; the molecular size of the amplification products was estimated by comparison with the marker 1 kb plus DNA ladder (GIBCO BRL, Life Technologies, Gaithersburg, MD, USA).

2.2 Statistical data analysis

Analysis of molecular variance (amova) was used to assess the partitioning of the genetic variance among different groups, and populations (Excoffier et al. 1992) using the program arlequin 2.000 (Schneider et al. 1997). amova divides the total variance into hierarchical partitions, among groups, among populations within groups and within populations. A test of permutation computes the statistical significance of each partition. The analyses were performed on haplotype frequencies only and for each analysis, 16 000 permutations were computed to obtain the significance levels of the variance.

A vector of presence/absence of M13 minisatellite markers was used to compute, with the Euclidean metric measurement, the matrix of genetic distances for each pair of isolates. amova procedure was used to estimate the variance components of M13 profiles, and to partition the variation among and within groups.

Table 1. **Isolates tested using M13 minisatellite**

Isolate	Code	Species	Host	Geographical origin
Pyr-5	970907 5.2.2.	*H. ab.*	*Abies alba*	Aucun, Pyrenees, France
Pyr-7	990219	*H. ab.*	*Abies alba*	Airege, Pyrenees, France
Fr-1	Del1	*H. ab.*	*Pinus sylvestris*	France
Fr-3	951025 H80	*H. ab.*	*Abies grandis*	Amance, France
Fr-4	951015 H81	*H. ab.*	*Abies alba*	Vosges, France
Fr-6	951130 H82	*H. ab.*	*Cedrus atlantica*	France
Fr-7	951130 H84	*H. ab.*	*Pseudotsuga menziesii*	France
It-1	920401 2ve	*H. ab.*[1]	*Pseudotsuga menziesii*	Vellano (PT), Italy
It-2	920401 3ve	*H. ab.*[1]	*Pseudotsuga menziesii*	Vellano (PT), Italy
It-3	931007 3.1	*H. ab.*	*Abies alba*	Valgrande (BL), Italy
It-9	950810 1.1ve	*H. ab.*[1]	*Abies alba*	Molveno (TN), Italy
It-14	931030 4.33	*H. ab.*	*Pinus nigra*	Serra San Bruno (VV), Italy
It-15	930921 1.4	*H. ab.*	*Abies alba*	Metato (FI), Italy
It-16	910910 7.1	*H. ab.*	*Abies alba*	Vallombrosa (FI), Italy
Gr-1	HL-2.1	*H. ab.*	*Abies cephalonica*	Parnon mountain, Greece
Gr-2	HT-4	*H. ab.*	*Abies cephalonica*	Taygetos mountain, Greece
Gr-3	HT-8	*H. ab.*	*Abies cephalonica*	Taygetos mountain, Greece
Gr-4	He-1	*H. ab.*	*Abies cephalonica*	Helikon mountain, Greece
Ch-5	950328.16	*H. ab.*	*Abies alba*	Zollikon, Switzerland
Ch-6	951218.1	*H. ab.*	*Abies alba*	Forch, Switzerland
Ch-7	951225.5	*H. ab.*	*Abies alba*	Forch, Switzerland
Sl-1	9210/4.2	*H. ab.*	*Abies alba*	Sneznik, Slovenia
Sl-4	9304/1.1	*H. ab.*	*Picea abies*	Trnovski, Slovenia
Pol-3	20029	*H. ab.*	*Abies alba*	Limanowa, Poland
Pol-4	20039	*H. ab.*[1]	*Abies alba*	Siemianice, Poland
Pol-7	20049	*H. ab.*[1]	*Abies alba*	Poland
Pol-9	20053	*H. ab.*[1]	*Abies alba*	Roztoczaski National Park, Poland
Pol-11	97006	*H. ab.*[1]	*Abies alba*	Ojcowski National Park, Poland
Bul-1	95 265.5	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Bul-3	95 269.6	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Bul-4	95 270.1	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Bul-5	95 271.4	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Bul-7	95 282.1	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Bul-8	95 284.2	*H. ab.*	*Abies alba*	Pirin Mountain, Bulgaria
Au-4	951115 1.4	*H. ab.*	*Picea abies*	Klein Krottenbachtal, Austria
Au-5	951115 1.5	*H. ab.*	*Picea abies*	Klein Krottenbachtal, Austria
Au-8	951115 2.3	*H. ab.*	*Picea abies*	Hensgstpass, Austria
Au-9	951115 2.4	*H. ab.*	*Picea abies*	Hensgstpass, Austria
Au-10	951115 2.5	*H. ab.*	*Picea abies*	Hensgstpass, Austria
Au-14	951115 3.2	*H. ab.*	*Picea abies*	Hensgstpass, Austria
Pol-1	20021	*H. p.*[1]	*Picea abies*	Suwaki, Poland
Pol-6	20048	*H. p.*[1]	*Abies alba*	Limanowa, Poland
Au-26	951115 7.2	*H. p.*	*Picea abies*	Paudorf, Austria
Au-21	951115 4.3	*H. a.*	*Pinus sylvestris*	Gars Fiecher, Austria
Au-24	951115 6.1	*H. a.*	*Pinus nigra*	Schonberg, Austria
Au-25	951115 6.2	*H. a.*	*Larix decidua*	Schonberg, Austria
Au-30	951115 8.1	*H. a.*	*Pinus sylvestris*	Paudorf, Austria

H. ab. = Heterobasidion abietinum; *H. p. = Heterobasidion parviporum*; *H. a. = Heterobasidion annosum s.s.*
[1]Heterokaryotic isolates.

A dendrogram was constructed using Nei and Li genetic distance (Nei and Li 1979) with Neighbor-Joining method (Saitou and Nei 1987). Analyses were conducted using phylogeny inference package software (Felsenstein 1993). The robustness of the inferred tree was evaluated by 500 bootstrap re-samplings.

3 Results

The electrophoretic profiles of the amplification products (Table 2) yielded a total of 32 markers with molecular weights between 300 and 12 000 bp, some of which were specific for one of the three *Heterobasidion* species. Some isolates of *H. abietinum*

Table 2. **Presence–absence (1/0) vector of *Heterobasidion s.l.* isolates tested with the M13 primer**

H. ab. Pyr-5	00000001001000000000110000000000
H. ab. Pyr-7	00000001001010000010100000000000
H. ab. Fr-1	00000001101010000010100000000000
H. ab. Fr-3	00001001001000000010100000000000
H. ab. Fr-4	00001001001000000010100000000000
H. ab. Fr-6	00001001001000000000100000000000
H. ab. Fr-7	00001001001010000000100000000000
H. ab. It-3	00001001001000000000100001010011
H. ab. It-1	00000001001000000000100001010001
H. ab. It-2	00000001001000000000100001010001
H. ab. It-9	00000101001000000000100001010001
H. ab. It-14	00000101001000000000100001010001
H. ab. It-15	00000101001000000000100001010001
H. ab. It-16	00000101001000000000100101010001
H. ab. Gr-1	01000001010000000111101000000000
H. ab. Gr-2	01001001010000000111101000000000
H. ab. Gr-3	01001001010000000111101000000000
H. ab. Gr-4	01000001010000000111101001000000
H. ab. Au-4	00000001000000000010100010000000
H. ab. Au-5	00000001001000001000100000000000
H. ab. Au-8	00000001000000000110010000000000
H. ab. Au-9	00000001000000001110110010110111
H. ab. Au-10	00000001000000001110100010001010
H. ab. Au-14	00000101000000000111010001000000
H. ab. Ch-5	00000101000000001000100100000001
H. ab. Ch-6	00001101001100101000100000000000
H. ab. Ch-7	00001001001100001000100100000000
H. ab. Sl-1	00000001001001101000100101000000
H. ab. Sl-4	00000001001001001110110101000000
H. ab. Pol-3	00001001000100000000100000000000
H. ab. Pol-4	00001101000100000000100000000001
H. ab. Pol-7	00001001000100000000100000000000
H. ab. Pol-9	00001111010100000000100000000000
H. ab. Pol-11	00001001010100000000100000000000
H. ab. Bul-1	00001001010000000110101000000000
H. ab. Bul-3	00000001010000000110101000000000
H. ab. Bul-4	00001001010000000110101000000000
H. ab. Bul-5	00001001010000000110101000000000
H. ab. Bul-7	00001001010000000110101000000000
H. ab. Bul-8	00001001010000000110101000000000
H. p. Pol-1	10001011000100000100000000000000
H. p. Pol-6	10001001000100000100000000000000
H. p. Au-26	10001001000100101100010000000000
H. a. Au-21	00111011000000111100000000000000
H. a. Au-24	00110011000100110100000000000000
H. a. Au-25	00010011000000110100000000011100
H. a. Au-30	00111011000100110100000000001000

H. ab. = *Heterobasidion abietinum*; *H. p.* = *Heterobasidion parviporum*; *H. a.* = *Heterobasidion annosum s.s.*

Table 3. **Results of analysis of molecular variance (AMOVA) showing the geographic distribution on the genetic variation of *Heterobasidion abietinum:* (a) AMOVA among four geographic regions (Italian peninsula, Balkan peninsula, central Europe and French Pyrenees); (b) AMOVA among four geographic regions (Italian peninsula, Balkan peninsula and French Pyrenees)**

Source of variation	Variance components	Percentage of variation	p-Value
a)			
Among groups	1.3	35.1	0.001
Among populations within groups	1.2	32.9	0.001
Within populations	1.2	32.0	0.001
b)			
Among groups	2.1	55.4	0.001
Among populations within groups	0.9	25.0	0.001
Within populations	0.7	19.6	0.001

showed identical banding patterns, but the profiles of the *H. parviporum* and *H. annosum* isolates all differed from each other. All isolates of *H. abietinum* had one marker in common; other markers were shared among isolates. The four isolates of *H. annosum* and the three isolates of *H. parviporum* had six and five monomorphic markers, respectively, although only small numbers of *H. annosum* and *H. parviporum* isolates were tested.

The Neighbor-Joining dendrogram (Fig. 1) separated isolates of the three *Heterobasidion* species into clusters. Isolates of *H. abietinum* were separated significantly from isolates of *H. annosum* and *H. parviporum* in the dendrogram. Isolates of *H. abietinum* clustered according to geographical origin, with the exception of those collected in the central Europe.

Analysis of molecular variance of the 40 isolates of *H. abietinum* collected in different geographical regions indicated that the percentage of genetic variation of this pathogen among four regions (Italian peninsula, Balkan peninsula, central Europe and French Pyrenees) was 35.1 ($p < 0.001$) with $\Phi_{st} = 68\%$, Table 3.

The dendrogram (Fig. 1) separated isolates from three parts of the Mediterranean basin: the Balkan peninsula, the Italian peninsula and the French Pyrenees, distinct clusters. Differences between these clusters, calculated with AMOVA, were significant ($p < 0.001$) and the percentage of genetic variation among these groups was 55.4 whit $\Phi_{st} = 80\%$. In contrast, the percentage of variation within group among populations and within populations increased to 25.0 and 19.6 ($p < 0.001$), respectively.

Isolates of *H. abietinum* collected in central Europe (Fig. 1) differed, however, constituting small clusters dispersed among and within the other three principal groups.

4 Discussion

The capacity of the M13 minisatellite as a primer to detect both isolate-specific and species-specific differences in *Heterobasidion* (STENLID et al. 1994) was confirmed. It was also found, consistent with KARLSSON (1993) that Neighbor-Joining clustering based on the M13 minisatellite profiles usually grouped the isolates according to geographic origin. *Heterobasidion abietinum* isolates collected from the Italian peninsula, Balkan peninsula and the French Pyrenees exhibited greater variation among regions (55.4%) than within populations (16.6.%). Similar results were reported by STENLID et al. (1994), who investigated genetic variations in local populations of *H. annosum* and *H. parviporum* collected at a certain distance from each other. LA PORTA et al. (1997) demonstrated that the variations between the Italian and north-European populations of *H. parviporum* were 71.1%.

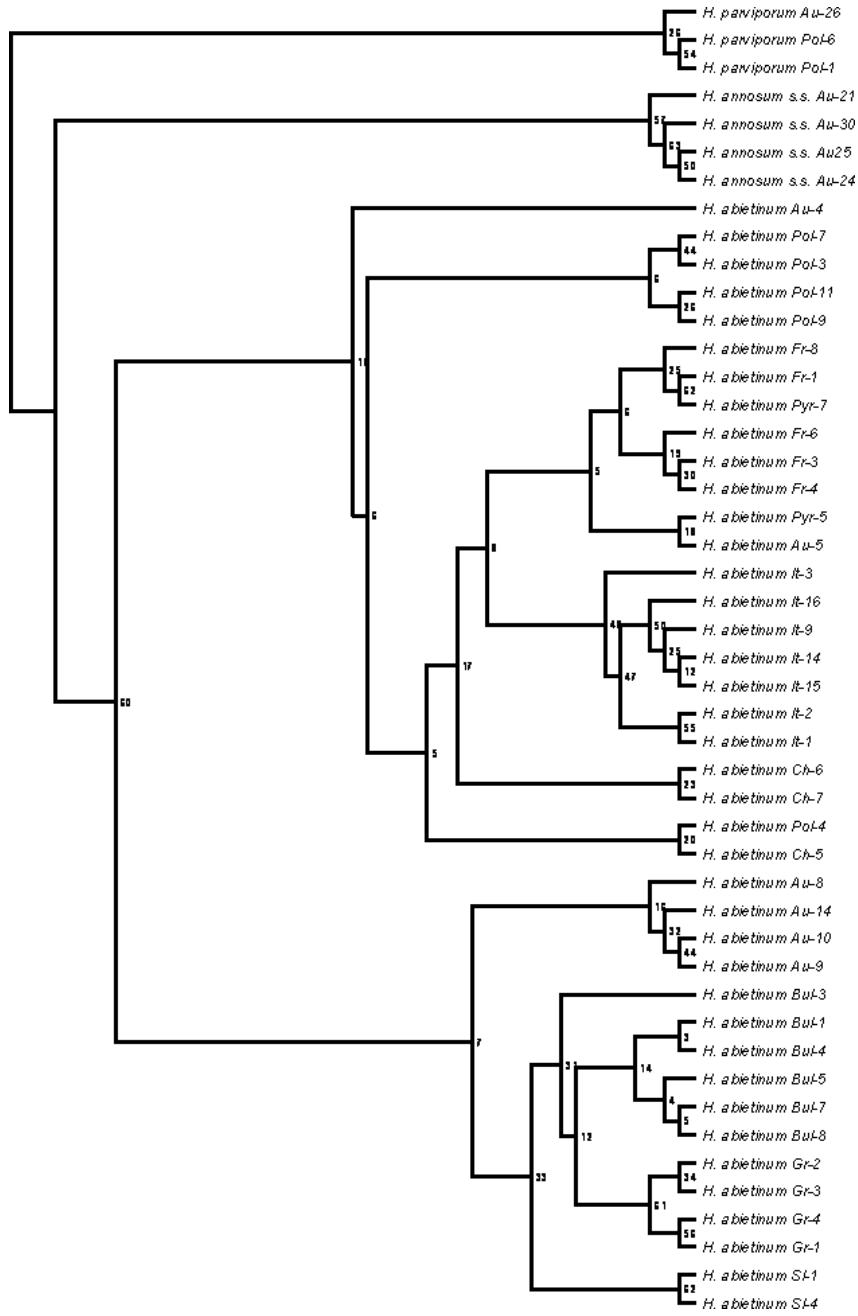

Fig. 1. Neighbor-Joining dendrogram for 47 European isolates of *Heterobasidion annosum*, *H. abietinum* and *H. parviporum*. Amplification profiles were obtained from PCR using the M13 minisatellite core sequence (for list of isolates see Table 1)

In the present study, limited genetic flow between populations was apparently due to the long distances between the regions; as a result, each population varied independently from the others, modifying its genetic heritage. The lack of variation found between individuals in a given geographical region may also have been due in part to the fact that the collection sites were not randomly distributed throughout the regions. Living spores of *Heterobasidion* may be dispersed in air for hundreds of kilometres, but the great majority of spores fall within a hundred or a few hundred metres of the spore source (Redfern and Stenlid 1998); hence, even relatively narrow geographic barriers may lead to populations of *Heterobasidion* becoming diversified.

A diversification into ecotypes similar to that found with *Heterobasidion* can also be seen in populations of the host tree *A. alba*: different ecotypes grow in the Apennines and in the Pyrenees (Parducci et al. 1996; Ziegenhagen et al. 1998). In the Alps and central Europe, where *A. alba* does not differentiate into ecotypes in this way, the differentiation of *H. abietinum* is also only slight. This may explain why differences between isolates were greater than differences between populations.

During the last Glacial age, Italy, the Pyrenees and the Balkan region were important refugia for many tree species, including *Abies, Picea* and *Pinus*. Populations of *Abies* survived in the Dinaric Alps, the Carpathian Mountains and southern Russia (Miller 1977; Bernetti 1998). *Abies alba* found refuge in Calabria (southern Italy), the Balkan region and the Pyrenees. When the continental ice sheet began to retreat again, *Pinus* was the first dominant conifer genus to advance outward into the wider Mediterranean area, where it achieved a large but fragmented distribution. *Picea abies* began to spread somewhat later, and *A. alba* later still. Northward migration enabled *Abies* species to re-colonize their present natural distribution, with a west to east migration in the Alps. The present distribution of *A. alba* in central Europe is due to spread of the Italian populations (Bernetti 1998), resulting in an absence of *A. alba* ecotypes and pathogen variation in the Alps and central Europe.

The relatively large genetic distances between *H. abietinum* populations in the Italian peninsula, the Greek peninsula and the Pyrenees underline the scarce gene flow between these regions and probably reflect the diversification that took place in both the pathogen and the host trees that grew in the refuge areas during the last ice age. The *Abies* forests in the Alps and central Europe are a more uniform population as regards both the host tree, *A. alba*, and its companion, *H. abietinum*.

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References

Bernetti, G., 1998: Selvicoltura speciale. Torino, Italy: UTET, 415 pp.

Dai, Y.-C.; Vainio, E. J.; Hantula, J.; Niemelä, T.; Korhonen, K., 2003: Investigations on *Heterobasidion annosum s.l.* in central and eastern Asia with the aid of mating tests and DNA fingerprinting. For. Pathol. **33,** 269–286.

Doğmuş-Lehtijärvi, H. T.; Lehtijärvi, A.; Korhonen, K., 2006: *Heterobasidion abietinum* on *Abies* species in western Turkey. For. Pathol. **36,** 280–286.

Excoffier, L.; Smouse, P.; Quattro, J., 1992: Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics **131,** 479–491.

Felsenstein, J., 1993: PHYLIP (Phylogen Inference Package), version 3.5c. Seattle: Department of Genetics, University of Washington (distributed by the authors).

Hogberg, N.; Stenlid, J., 1999: Population genetics of *Fomitopsis rosea* – a wood-decay fungus of the old – growth European taiga. Mol. Ecol. **8,** 703–710.

Hogberg, N.; Holdenrieder, O.; Stenlid, J., 1999: Population structure of the wood decay fungus *Fomitopsis pinicola*. Heredity **83,** 354–360.

Karlsson, J.-O., 1993: Genetic variation in *Heterobasidion annosum* detected with M13 fingerprinting and ribosomal DNA probes. Exp. Mycol. **18,** 48–56.

Korhonen, K.; Dai, Y.-C., 2005: Genetically identified taxa of *Heterobasidion* and their distribution in Eurasia. In: Proc. 11th Int. Conf. Root and Butt Rots. Ed. by Manka, M.; Łakomy, P., pp. 57–63.

Korhonen, K.; Capretti, P.; Karjalainen, R.; Stenlid, J., 1998: Distribution of *Heterobasidion annosum* intersterility groups in Europe. In: Heterobasidion annosum. Biology, Ecology, Impact and Control. Ed. by Woodward, S.; Stenlid, J.; Karjalinen, R.; Huttermann, A. Oxon: C.A.B. International, pp. 93–104.

La Porta, N.; Capretti, P.; Kammiovirta, K.; Korhonen, K.; Karjalainen, R., 1997: The relatedness of the Italian F intersterility group of *Heterobasidion annosum* with the S group, as revealed by RAPD assay. Mycol. Res. **101,** 1065–1072.

Łakomy, P., 1996: F group of *Heterobasidion annosum* found in Poland. Eur. J. For. Path. **26,** 217–222.

Miller, C. N., 1977: Mesozoic conifers. Bot. Rev. **43,** 217–280.

Munda, A., 1994: Preliminary report on the distribution of *Heterobasidion annosum* intersterility groups in Slovenia. In: Proc. 8th Int. Conf. Root and Butt Rots, Wik, Sweden and Haikko, Finland. 9–16 August 1993. Ed. by Johansson, M.; Stenlid, J. Uppsala: Swed. Univ. Agric. Sci., pp. 272–273.

Nei, M.; Li, W. H., 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. PNAS **76,** 5269–5273.

Parducci, L.; Szmidt, A. E.; Villani, F.; Wang, X. R.; Cherubini, M., 1996: Genetic variation of *Abies alba* in Italy. Hereditas **125,** 11–18.

Redfern, D. B.; Stenlid, J., 1998: Spore dispersal and infection. In: Heterobasidion annosum. Biology, Ecology, Impact and Control. Ed. by Woodward, S.; Stenlid, J.; Karjalinen, R.; Huttermann, A. Oxon: C.A.B. International, pp. 105–124.

Sánchez, M. F.; Capretti, P.; Calzado, C.; Navarro, R.; Trapero, A., 2005: Root rot disease on *Abies pinsapo* in southern Spain. In: Proc. 11th Int. Conf. Root and Butt Rots. Ed. by Manka, M.; Łakomy, P., pp. 220–223.

Saitou, N.; Nei, M., 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4,** 406–425.

Santini, A.; Capretti, P., 2000: Analysis of the Italian population of *Ceratocystis fimbriata* f.sp. *platani* using RAPD and minisatellite markers. Plant Pathol. **49,** 461–467.

Santini, A.; Montaghi, A.; Vendramin, G. G.; Capretti, P., 2005: Analysis of the Italian Dutch Elm Disease Fungal population. J. Phytopathol. **153,** 1–7.

Schneider, S.; Kueffer, J. M.; Roessli, D.; Excoffier, L., 1997: Arlequin ver 1.1: a Software for Population Genetic Data Analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Smith, D. R.; Stanosz, G. R., 1995: Confirmation of two distinct populations of *Sphaeropsis sapinea* by in the North Central United States using RAPDs. Phytopathology **85,** 699–704.

Stenlid, J.; Karlsson, J.-O.; Hogberg, N., 1994: Intraspecific genetic variation in *Heterobasidion annosum* revealed by amplification of mini satellite DNA. Mycol. Res. **98,** 57–63.

Vasiliauskas, R.; Stenlid, J., 1997: Population structure and genetic variation in *Nectria fuckeliana*. Can. J. Bot. **75,** 1707–1713.

Vasiliauskas, R.; Stenlid, J., 1998: Population structure and genetic variation in *Cilindrobasidion evolvens*. Mycol. Res. **102,** 1453–1458.

Woodward, S.; Stenlid, J.; Karjalainen, R.; Hütterman, A., 1998: *Heterobasidion annosum*. In: Biology, Ecology, Impact and Control. Ed. by Woodward, S.; Stenlid, J.; Karjalinen, R.; Huttermann, A. Oxon: C.A.B. International, 589 pp.

Ziegenhagen, B.; Scholz, F.; Madaghiele, A.; Vendramin, G. G., 1998: Hypervariable chloroplast microsatellites as markers for paternity analysis in *Abies alba*. Can. J. For. Res. **28,** 317–321.