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Long-distance spore dispersal in wood-inhabiting Basidiomycetes

Nils Hallenberg and Nicolas Küffer

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Eight species of wood-inhabiting basidiomycetes (*Laurilia sulcata, Peniophora aurantiaca, Resinicium bicolor, Scytinostroma galactinum, Terana caerulea, Trichaptum abietinum, T. biforme* and *T. fuscoviolaceum*) were used in a spore-trapping test to evaluate their individual ability for long-distance spore dispersal. Petri dishes with single spore mycelia were used as baits. In the experiment, carried out at the Botanical Institute in Göteborg, spores from the air were regularly captured. Surprisingly, spores were captured from species whose nearest known natural occurrence was located quite far from Göteborg. The closest population of *Peniophora aurantiaca* is about 1000 km south of Göteborg. The results from this experiment support the hypothesis that fungal spores are widely and efficiently dispersed. Such a broad and extensive dispersal ability is of vital importance, especially for wood-inhabiting species which are highly dependent on a substrate which is only temporarily available.

N. Hallenberg, Botanical Institute, Göteborg University, Box 461, S-405 30 Göteborg, Sweden. E-mail: nils.hallenberg@systbot.gu.se. – N. Küffer, Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland. Present address: Laboratoire de microbiologie, Université de Neuchâtel, Case Postale 2, CH-2007 Neuchâtel, Switzerland.

Introduction

Species of wood-inhabiting basidiomycetes are generally distributed over wide geographical areas. Within the temperate zone of the Northern Hemisphere a circumpolar distribution has been verified for many species, when the species concept is defined by intercompatibility or basidiome monomorphism (Hallenberg 1995). How this could be accomplished is still a matter of speculation. It is true that intercompatibility and basidiome monomorphism is not sufficient evidence for gene flow over intercontinental distances among recent populations. A more likely explanation for such a wide distribution could be found in biohistorical events in combination with a slow rate in species evolution (Hallenberg 1995). Even persisting intercompatibility between strains from different continents may be of relict nature (Petersen & Hughes 1999). Nevertheless, within large regions – like parts of continents – a coherent distribution is frequently found and reasonably, this must be connected to an efficient dispersal within recent populations and a gene flow mediated by airborne spores.

In local populations spore dispersal also has to be efficient to allow populations to survive. The appropriate substrate for these fungi, decaying wood, will always have a limited temporal availability, which makes recurrent new establishments of spores a frequent event. Experiences from species inventories based on the collecting of basidiomata, show that wood-fungi in general are scattered in the local landscape and individual species rarely occur in high numbers. Sampled species are typically recorded once or twice.

Species-specific spore-trapping experiments, with monokaryotic colonies as baits, were introduced by

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Adams et al. (1984) and Williams et al. (1984). The technique depends on the assumption that conspecific spores of a complementary mating type are able to pair with a precultivated monokaryotic mycelium, and as a result a secondary mycelium with clamp connections is formed. Several successful investigations have repeated this method (e.g. Vilgalys & Sun 1994, Nordén & Larsson 2000), which is very simple but also one of the few available methods to detect airborne fungal spores on a species-specific level. In this way, occurrence of conspecific spores will be recorded even if their density is extremely low.

In the present study some of the species selected for the spore-trapping experiment do not occur within 800 km or more from the bait, while others frequently occur in the nearby surroundings. If spores from such distant species are frequently recorded in these experiments, we have a good indication that their absence or rarity in the local environment is dependent on other factors than spore dispersal.

Material and methods

From the Culture Collection at University of Göteborg (FCUG), 8 species and a total of 21 different monokaryotic strains were selected for spore trapping experiments (Table 1). Reasons for selecting these particular species are that 1) they have a wide geographical distribution, being collected from different parts of the Northern Hemisphere, 2) they are easily observed and identified and should hardly escape any serious inventory, 3) some of the species have a nearest known location quite far away from the spore trapping site (Göteborg). The map (Fig 1) indicates regions where their closest known occurrencies are found in Europe. The flora of wood-inhabiting basidiomycetes is particularly well known in northern Europe and distribution records of the selected species are, therefore, reliable.

Actively growing monokaryotic mycelia were transferred to 9 cm Petri dishes filled with 1.5 % malt extract agar (MEA). They were incubated for four to six weeks at room temperature until the mycelia had completely covered the dishes. Thereafter, the Petri dishes were placed for 24 hours on the roof of the Botanical Institute, Göteborg University, with their lids removed. For each strain three replicates were used and, as a negative control, one additional dish was left with its lid closed. After being exposed to atmospheric spore rain, the Petri dishes were incubated for four to five weeks at 14°C to allow for the possible growth of secondary mycelia. The lower temperature was chosen in order to discourage development of moulds. The experiment was repeated six times for the eight species, between October 4 and November 8, 1999. When available, different single spore isolates of the same species were used at different exposure events (Table 2).

Finally, mycelia were checked for clamp connections as a verification of successful pairings. The existence of clamped hyphae indicates that effective dikaryotisation and nuclear migration had taken place. Each dish was checked at three spots of the mycelium to make sure that clamped hyphae were present. The negative controls were checked for absence of clamp connections and the original mycelia from the fungal culture collections were checked once more.

As an additional control a di-mon test (Boidin 1980, Hallenberg 1984) was performed with subcultures from all successfully established secondary mycelia. In this test, a secondary mycelium obtained after spore-trapping was crossed with the original

Table 1. List of selected cultures. Culture number is followed by number of single-spore (SS) mycelia used in this study, species name, substrate and location of the original specimen. Compatibility between selected SS-cultures indicated.

FCUG 2318, SS-2, 6/ *Laurilia sulcata* (Burt)Pouz./ Russia, Far East. SS-2 compatible with SS-6.
FCUG 2564, SS-1, 3, 9/ *Peniophora aurantiaca* (Bres.)Höhn. & Litsch./ *Alnus*/ Canada, BC. SS-1 compatible with SS-3, both incompatible with SS-9.
FCUG 2606, SS-2,3,6/ *Resinicium bicolor* (Fr.)Parm./ *Abies*/ Germany, Bayern. SS-2, 3 compatible with SS-6.
FCUG 1232, SS-1, 2,9/ *Scytinostroma galactinum* (Fr.)Donk/ *Tilia*/ Sweden, Uppland. SS-1, 2 compatible with SS-9.
FCUG 2713, SS-1, 2/ *Scytinostroma galactinum* (Fr.)Donk/ *Pterocarya*/ Russia, Krasnodar. SS-1 compatible with SS-2.
FCUG 1816, SS-3/ *Terana caerulea* (Fr.)Kuntze/ deciduous wood/ France, Provence.
FCUG 2581, SS-1, 2, 3, 8/ *Trichaptum abietinum* (Fr.)Ryv./ *Picea*/ Finland, Etelä-Häme. SS-1, 8 compatible with SS-2, 3.
FCUG 2730, SS-1, 2/ *Trichaptum biforme* (Fr.)Ryv./ *Carpinus*/ Russia, Krasnodar.
FCUG 1894, SS-5/ *Trichaptum fuscoviolaceum* (Fr.)Ryv./ *Pinus*/ Sweden, Västergötland

Fig. 1. Map of parts of Europe showing the location of the spore-trapping experiment (Göteborg) and, roughly, the closest known occurrence of species supposed to have fertilised the haploid baits via long-distance spore dispersal.

monokaryotic strain from the culture collection. If the monokaryotic strain becomes clamped as a result of the di-mon test, we have a good indication that conspecific and viable spores were distributed to the Petri dish during the time of exposure. A source of error in this experiment could be the transfer of asexual propagules between the Petri dishes. However, the only species with asexual spore production among the selected species are *Resinicium bicolor* and *Laurilia sulcata*. The first species is naturally occurring in the vicinity of the institute while for the second species only one mating type was used as tester at each exposure. Theoretically, the dispersal of hyphal fragments between different single spore isolates of the same species could be an unwanted cause to dikaryotisation. However, dikaryotisation was detected for all species from exposure events when only a single mating type was represented (Table 2). It is therefor unlikely that internal crossing could be an explanation to the results presented here.

Among the studied species, *Resinicium bicolor* and *Trichaptum abietinum* are abundant near the Botanical Institute. *T. fuscoviolaceum* is considerably less frequent but still recorded in this area (within a few kilometres).

Laurilia sulcata and *Scytinostroma galactinum* are both recorded in Sweden but at a considerable distance from Göteborg. *L. sulcata* is a northern species in Europe with the closest populations in northern Dalarna and south-eastern Norway, about 400 km north of Göteborg. *S. galactinum* is a rare species in south and central Sweden with very few findings during the last century (Larsson 1997). The species is more common in southern Finland, at least 800 km from Göteborg.

Trichaptum biforme is a south-eastern European species, its nearest recorded occurrence being south-eastern Finland, almost 1000 km E of Göteborg (Kotiranta & Niemelä 1996). *Peniophora aurantiaca* is in Europe restricted to *Alnus viridis* as host (Küffer & Senn-Irlet 2000), and has its most northern localities in southern Germany and in Slovakia, approx. 1000 km south of Göteborg. Finally, *Terana caerulea* is recorded as an isolated population in south-western Norway (400 km) and more frequently towards the south and south-west, approx. 800 km from Göteborg (Krieglsteiner 2000).

Results and discussion

The results from the spore trapping experiment are summarised in Table 2. It follows that the monokaryotic strains from all studied species became

Table 2. Results of the spore trapping during 6 different exposure events. For each species the following symbols have been used: cates, "0" = missing data. Under "success" is given the number of exposure events when pairing was noted divided by the total

Exposure (nr/date):	1 (4/10/99)	2 (7/10/99)	3 (11/10/99)
Species: / weather:	rain, windy	rain, calm	no rain, storm
Laurilia sulcata FCUG 2318, SS-2	0	-	-
Laurilia sulcata FCUG 2318, SS-6	0	0	0
P. aurantiaca FCUG 2564, SS-1	-	-	0
P. aurantiaca FCUG 2564, SS-3	0	0	-
P. aurantiaca FCUG 2564, SS-9	0	-	-
Resinicium bicolor FCUG 2606, SS-2	0	-	-
Resinicium bicolor FCUG 2606, SS-3	0	0	**
Resinicium bicolor FCUG 2606, SS-6	-	-	*
Scyt. galactinum FCUG 2713, SS-1	**	*	0
Scyt. galactinum FCUG 2713, SS-2	0	0	0
Scyt. galactinum FCUG 1232, SS-1	0	0	*
Scyt. galactinum FCUG 1232, SS-2	*	**	0
Scyt. galactinum FCUG 1232, SS-9	0	0	0
Terana caerulea FCUG 1816, SS-3	*	*	-
Trich. abietinum FCUG 2581, SS-1	0	0	*
Trich. abietinum FCUG 2581, SS-2	*	*	0
Trich. abietinum FCUG 2581, SS-3	0	0	0
Trich. abietinum FCUG 2581, SS-8	0	0	0
Trich. biforme FCUG 2730, SS-1	*	-	-
Trich. biforme FCUG 2730, SS-2	0	0	0
T. fuscoviolaceum FCUG 1894, SS-5	0	-	*

dikaryotized at two or more exposure events. All negative controls, where the lid was attached to the Petri dish during exposure, remained unclamped and in all tests for conspecificity with di-mon pairings, the original monokaryotic strain had been dikaryotised by the clamped mycelia from the Petri dishes. Each time we tried to catch spores, we also noted the local weather conditions. Spore trapping seemed to be successful on both clear and rainy days, with stormy to completely calm periods. Wind directions were variable during the period. To evaluate the effect of weather much more data is needed as well as a different sampling strategy.

From Table 2 it also follows that different monokaryotic strains of the same species differ from each other in their mating abilities. Some strains seem to get clamped more easily than others on exposure to airborne spores. For instance, in strain number FCUG 2606, SS-3 (*Resinicium bicolor*) secondary mycelium was formed more frequently than in SS-2 and SS-6. Still, the number of samples is too low to allow an appropriate evaluation of this phenomenon.

Spore-trapping studies, like the present one, involve some obscurities. As shown by Nordén & Larsson (2000) in their studies on *Phlebia centrifuga* P. Karst., there is a high deposit ratio of spores close to basidiomata in nature. Farther away, however, spore concentration decreases considerably, but it does not seem to reach zero. The concentration may be high enough to allow a formation of secondary mycelia in spore-trapping baits, but there is still uncertainty about the minimum spore concentration needed for efficient establishment in nature. Efficient establishment could depend on the duration of 'spore rain' which is one reason why this study was based on repeated experiments during a prolonged period. Finally, it is a common misconception that the density of airborne spores is logarithmically decreasing with distance from spore producing basidiomata. This may be approximately valid in close vicinity to the source, but farther away turbulence in the air will divide a spore cloud into "spore packages" which may be dispersed long distances in an unpredictable way (Hirst & Hurst 1967).

"-" = no pairing, "*" = successful pairing only in one of the three replicates, "**" = successful pairing in two or all three repli-
number of exposure events for the species concerned.

4 (18/10/99)	5 (25/10/99)	6 (8/11/99)	Success rate per species
no rain, calm	rain, calm	no rain, calm	
0	0	0	2/4
0	**	*	
0	-	0	1/7
**	0	0	
0	0	0	
-	0	0	4/10
*	**	0	
0	-	0	
-	0	0	7/10
0	*	0	
0	**	0	
-	0	0	
-	0	0	
-	-	0	2/5
-	0	0	7/8
0	*	0	
0	0	*	
**	0	**	
-	-	0	3/7
0	*	*	
-	0	*	2/4

The purpose of this study was to find out if airborne dispersal by spores could be detected far away from the observed ranges of species distribution. Even if the species selected are easily identified there is a possibility that some basidiomata may occur more closely to Göteborg in nature than believed earlier. Such "undiscovered" basidiomata are, however, most probably few and their contribution insignificant to the spore content in air. It seems most likely that the vital spores recorded in the air in this experiment originate from coherent populations of the species and consequently released from a distance of up to 1000 km from the spore-trapping plot in Göteborg. Under this assumption it can be concluded that deficiency in airborne spore dispersal is not a factor which delimits a species range. A determining factor is more likely related to competition between established mycelia or, in other words, outside its range any particular species is not competitive enough to allow basidiome formation, or only rarely so. Consequently, we could expect to find non-fructifying mycelia – or various derivatives from such mycelia – from many more species than usually found when only basidiomata are recorded.

So far, there is no method for extensively registering the biodiversity of mycelia in nature but several important observations support the above mentioned hypothesis.

1. Among basidiomycetes, border lines for species ranges are generally very diffuse when not specifically host-restricted.

2. A great number of species occur rarely but over wide areas and when recorded there is typically just a single finding at one place. In the present study, spores from *Scytinostroma galactinum* were captured but there are so far only three records (basidiomata) of this species from Sweden.

A scattered or rare occurrence is easily understood when assuming that many more "invisible" mycelia are present in nature and that basidiome production could be the effect of specific environmental conditions appearing irregularly over the years.

There could be some doubt about the relevance of these spore-trapping results for real establishments in

nature. Moreover, in most cases two compatible mycelia are necessary for successful development and fruiting. However, establishment of haploid mycelia as a result of airborne spore dispersal over fairly long distances is a well known phenomenon among plant pathogenic fungi. Successful pairing in nature is probably best explained as a spore-trapping event (Hallenberg 1995). Already established haploid mycelia may be fertilised by haploid spores which arrive later. If we assume that the receptive phase for a haploid mycelium is distinctly prolonged, the chances for successful pairing increases greatly. Unfortunately, it is usually just as difficult to detect haploid mycelia as their dikaryotic counterpart. In *Heterobasidion annosum* (Fr.) Bref., however, haploid mycelia can be recognised by their oedocephaloid conidiophores and Stenlid (1994) detected haploid mycelia on a spruce stump surface four months after dissemination. Kallio (1970) used the possibility to detect haploid establishments of this species in a kind of spore trapping where slices of fresh cut spruce stems were exposed to the air out in the Sea. A few haploid establishments were detected and these were supposed to be the result of fairly long distance dispersal (across the Baltic Sea).

A problem with spore trapping is undoubtedly that a number of unwanted spores are caught during exposure. Several of these moulds may interfere with the monokaryotic bait and threaten its survival. It is possible for haploid mycelia to resist growth of spores from other species if precultivation has been well executed, with the mycelium covering the whole surface of the Petri dish. A low incubation temperature is also efficient in retarding mould growth. The resistance towards moulds was particularly strong in some of the studied species, viz. *Laurilia sulcata*, *Resinicium bicolor*, and *Trichaptum* spp. This high resistance is probably related to establishment strategies in nature for those species. In studies on interspecific interactions between wood-inhabiting basidiomycetes (Holmer et al. 1997, Holmer & Stenlid 1997), these species were found to be strong competitors.

A further interesting observation was the difficulties of certain monokaryotic strains to get paired, while other strains of the same species were favoured. This has been interpreted as "mating selection" by Liou et al. (1995) and also noted by Nordén & Larsson (2000). The phenomenon is probably a consequence of different abilities by the monokaryotic baits to promote nuclear migration upon pairing and is a question for future studies.

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