The distribution of nuclei in a natural population of the basidiomycete Schizophyllum commune

Source: http://www.rbgsyd.nsw.gov.au/

Abstract:

From laboratory studies it is known that there are three different mechanisms (mon-mon mating, di-mon mating and donation of a nucleus by a basidiospore) which play a role in the distribution of nuclei in the basidiomycete *Schizophyllum commune*. How and if those mechanisms also operate in nature remains unknown. The aim of this study was to reveal this distribution of nuclei in natural populations of the fungus to deduce which mechanisms are the driving force in nuclear distribution. In total 60 dikaryons were isolated from mushrooms growing on three tree branches. RPB2 sequences were used to group identical dikaryons and the similarities between the groups were investigated using mating type analysis. It was found that multiple dikaryons with unique nuclei can exist on a single branch which means that different mycelia are present, a prerequisite for mon-mon and di-mon interactions. It was also argued that selection is more likely to happen on the level of nuclei and less on the level of dikaryons.

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Introduction

A high genetic diversity allows an organism to cope with a range of circumstances and also allows an organism to adapt easily to new situations. The most important means to gain high genetic diversity is sexual reproduction. Sexual reproduction can happen in various ways. In some organisms, like plants and sessile marine organisms, large amounts of gametes are released into the open. In this case no actual mating takes place. However, in many other organisms, physical contact between individuals is necessary in order to exchange genetic material (Chen and Mcdonald 1996). When mating takes place, two roles can be distinguished, namely the male and female role. Even in hermaphroditic species it is possible to distinguish between those roles on gamete level (the egg cell and sperm cell)(Anderson and Kohn 2007). In basidiomycetes (mushroom forming fungi) also sexual reproduction takes place, but the different sexual roles are not as clear when they are compared to organisms from other divisions. To understand why, the lifecycle of basidiomycota has to be considered.

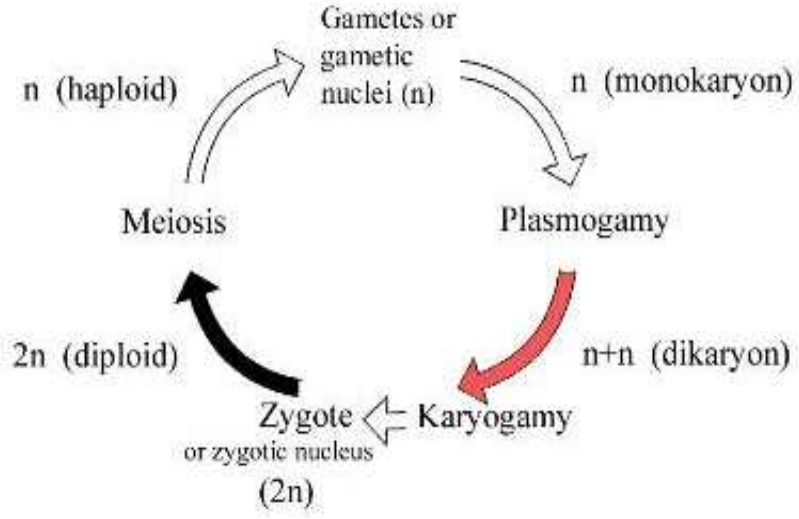


Figure 1 Life cycle of basidiomycota

Source: http://www.mansfield.ohio-state.edu/

The life cycle of the basidiomycetes is depicted in figure 1. A fungus starts as a haploid monokaryon. When mycelia of two different monokaryons meet cells fuse (plasmogamy) and reciprocal nuclear exchange can take place, resulting in one dikaryotic mycelium. It is not until the basidia are formed that actual nuclear fusion (karyogamy) takes place. Consequently, the fungus is dikaryotic throughout the largest part of its life (Anderson and Kohn 2007).
An interesting fact is that during dikaryotization in *S. commune* nuclei migrate from the zone of fusion throughout the entire mycelium into each receiving monokaryon. In contrast, cytoplasmic components are *not* transferred to other cell compartments. This mating thus

results in a mycelium with one single genotype but a mosaic for cytoplasm (see figure 2) (Aanen *et al.* 2004).

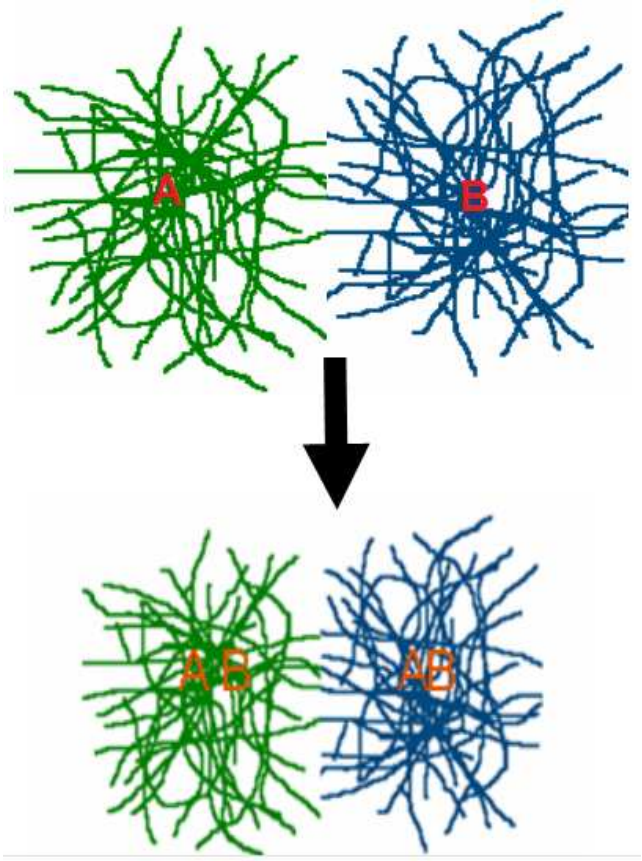


Figure 2. Two monokaryotic hyphae are about to meet. Both monokaryons have different nuclei (A and B) and a different cytoplasmic background (indicated by the bleu and green color). Upon cellular fusion nuclei are exchanged, but the cytoplasmic elements remain in their original mycelium. This results in a mycelium with one nuclear genotype (a dikaryon with nuclei A and B) which is mosaic for cytotype (the bleu and green colors).

The distinct sexual roles can be defined by the differences in size of gametes. When two monokaryons exchange nuclei, the fungus can be considered hermaphroditic, since they both perform a male role (the donation of nuclei) and a female role (the acceptance of nuclei and the provision of cytoplasm). However, as soon as the mycelium is a dikaryon the female function is lost since cell compartments can not contain more than two nuclei. But the mycelium never loses the male role since a dikaryon is able to donate one of its nuclei to a monokaryotic mycelium. This phenomenon was first described by Buller (1930) and hence called the buller phenomenon (Quintanilha 1937).

Three different ways of fertilization have been described in filamentous basidiomycetes; 1. Two monokaryotic mycelia mate and exchange nuclei, 2. One monokaryon receives a nucleus from a dikaryon (the Buller phenomenon) and 3. A monokaryon is fertilized by a spore. In the latter case only one mycelium will exist because the spore cannot produce a mycelium as all space is already occupied by the existing mycelium. However, the spore is able to donate its nucleus to the monokaryotic mycelium (Anderson and Kohn 2007). Even though these three forms of mating have been found in laboratory experiments, it is unknown if and how often these different interactions happen in nature. The focus of this study is to investigate how mating occurs under natural conditions and how the different mating mechanisms contribute to the distribution of nuclei. This was done by examining three trunks of wood containing *S. commune* fruiting-bodies. By determining both the nuclear genotype and cytotype of all fruiting-bodies it is possible to deduce how nuclei migrated through the different mycelia and thus obtain a better understanding of mating in nature. See table 1 for hypotheses.

Table 1. Possible ways of dikaryotization:

End results:	One cytotype within the mycelium	multiple cytotypes within the mycelium
One nuclear genotype within the mycelium	1. All fruiting bodies originate from one individual monokaryon dikaryotized by a single basidiospore	2. Fruiting bodies originate from two individual monokaryons which mated (mon – mon) and became one dikaryon in two cytotypes
multiple nuclear genotypes within the mycelium	3. An initial monokaryon is allowed to grow before multiple simultaneous fertilization events on different sites on the mycelium	4. Dikaryotization events happen simultaneously on different places. Depending on type of cross (di-mon, mon-mon or fertilization by basidiospore) the cytotypes to genotypes may be skewed.

The first scenario, in which all fruiting bodies have the same genotype and cytotype, is unrealistic due to the high number of airborne basidiospores; monokaryons placed outside in an open petri-dish are invariably dikaryotized (Anderson and Kohn 2007), even outside the natural range of the organism (James *et al.* 2001). Due to this high number of basidiospores it seems unlikely to find only one cytotypic individual of S. commune on a suitable piece of wood.
For the same reason, the second scenario is also unrealistic.
The third situation implies the monokaryon has plenty of time to grow before it is fertilized. When the monokaryon is fertilized it has to happen at different sites on the mycelium simultaneously. It seems that the fourth situation is most likely under natural conditions.

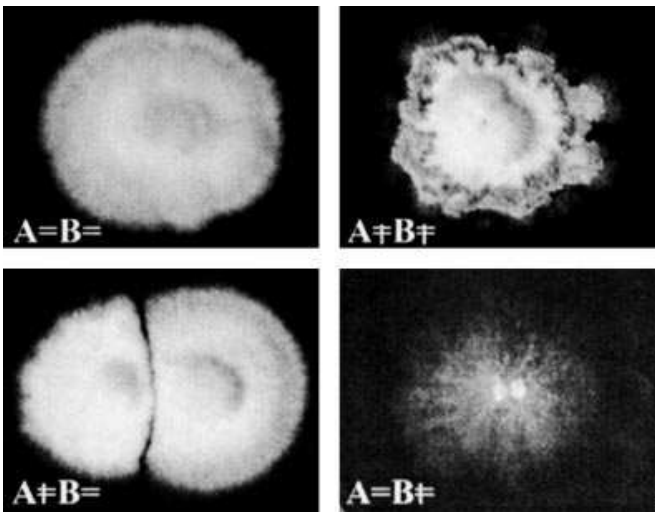


Figure 3. Reaction of mon-mon matings due to mating compatibility determined by mating type (Kothe 1999)

Textbox 1. Mating compatibility
Mating compatibility in *S. Commune* is regulated genetically. Upon mating, four different reactions can be distinguished, making this species tetrapolar (see figure 3). The four different reactions can be attributed to the two mating type factors: A and B. Both factors consist of two loci, namely Aα, Aβ, Bα and Bβ. Nine different allelic forms of Aα have been found and it is estimated that Aβ contains 32 different forms. The two different B loci both have nine different forms. All different combinations add up to 23.328 theoretical different mating types for *S. commune*. This high number promotes outbreeding and therefore increases heterozygosity, because spores are compatible with only 25% of their siblings, but with most non-siblings (Kothe 1999).

By revealing both the nuclear and mitochondrial genotypes of all mushrooms on a single piece of wood it is possible to answer multiple questions. The appearance of fungal mosaics (the appearance of multiple cytotypes within a genotype or genotypes within a cytotype) for instance would indicate that haploid monokaryons might have considerable time to grow and that during a fertilization event migration of different nuclei from spores or dikaryons can happen simultaneously within a mycelium with a single cytotype. The genomic constitution of multiple individuals of *S. commune* on a tree trunk may, besides answers to those questions, reveal other aspects of mating which have important evolutionary consequences which are not easily observed in nature.

Materials and methods

Sample extraction and DNA isolation
Two tree logs (tree 30 and 32; ~5cm diameter and ~1.5m long) and a smaller branch (tree 31; ~2cm diameter and 34cm long) containing *S. commune* were collected in the surroundings of Wageningen, the Netherlands (see appendix for pictures). 24 mushrooms were isolated from trees 30 and 32 and 12 mushrooms from the smaller tree 31. Small inoculums were isolated from the stem of the fruiting bodies growing on the logs and cultivated on minimal medium (Dons *et al.* 1979) at 27 ºC. Fresh mycelium for DNA extraction was grown for three days on cellophane covered plates to harvest mycelia (~15g) from the medium. DNA was extracted using the standard phenol chlorophorm protocol (Sambrook *et al.* 1989) after which the nucleic acids were dissolved in 50 µl purified water and finally treated with RNase (30 min at 37 ºC).

Relating mycelia: Genotyping and mating type analysis
To reduce the amount of crosses in the mating type analysis, the samples were first put in groups based on their nuclear genotype, since it is very likely that a single tree contains many clones. As a result it was not necessary to use every sample in the mating type analysis, but one representative per group instead (see fig. 5). To genotype the nuclei of the mycelia several sequences were tried. The region between Ef595F and EF1160R (Kauserud and Schumacher 2003) on the elongation factor gene was not variable enough. Two sequences on the RPB2 (second largest subunit ribosome polymerase gene) regions were tried as well; The region between fRPB2-7CF and fRPB2-11aR (Liu *et al.* 1999) was not informative, but the region between bRPB2-6F and bRPB2-7.1R proved to be variable enough to discriminate between the different individuals. Primers were synthesized for PCR amplification (Matheny 2005)(RPB2-6F: 5'-TGG GGK WTG GTY TGY CCT GC-3' and RPB2-7.1R: 5'-CCC ATR GCY TGY TTM CCC ATD GC-3').

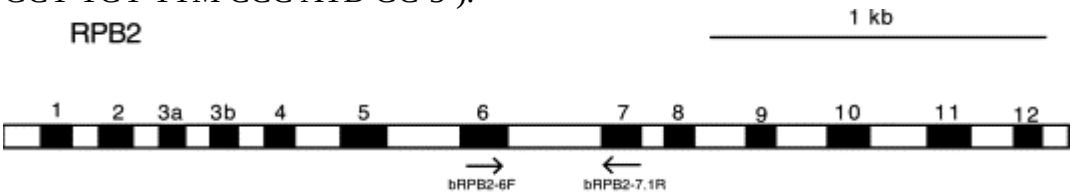


Figure 4. Primer location on the RPB2 gene (Matheny 2005).

PCR reaction mixes (18 µL in total) (Promega Corp., Madison, Wisconsin) contained 5 µL 5x PCR buffer (GoTaq green), 1 µL 10 mM dNTP, 0.13 µL Taq polymerase (GoTaq) (5U/l), 10.87 µL MQ, 0.5 µL 10 µM of each PCR primer and 2µL DNA 10x. The amplification program included an initial step of 95 C for 4 min, followed by 34 cycles of 95 C for 60s, 50 C for 60s, a ramp of 0.3 C per sec to 72 C and 72 C for 1 min. The program was concluded by one step 72 C for ten minutes and finally the temperature was hold at 4C. The PCR product

was purified using QIAquick Pcr purification kit by QIAgen. The pcr product was air dried and sent to Eurofins for sequencing using the same primers used for the pcr procedure. The sequences were analyzed and clustered using SeqMan from the software package DNAstar (lasergene 8).

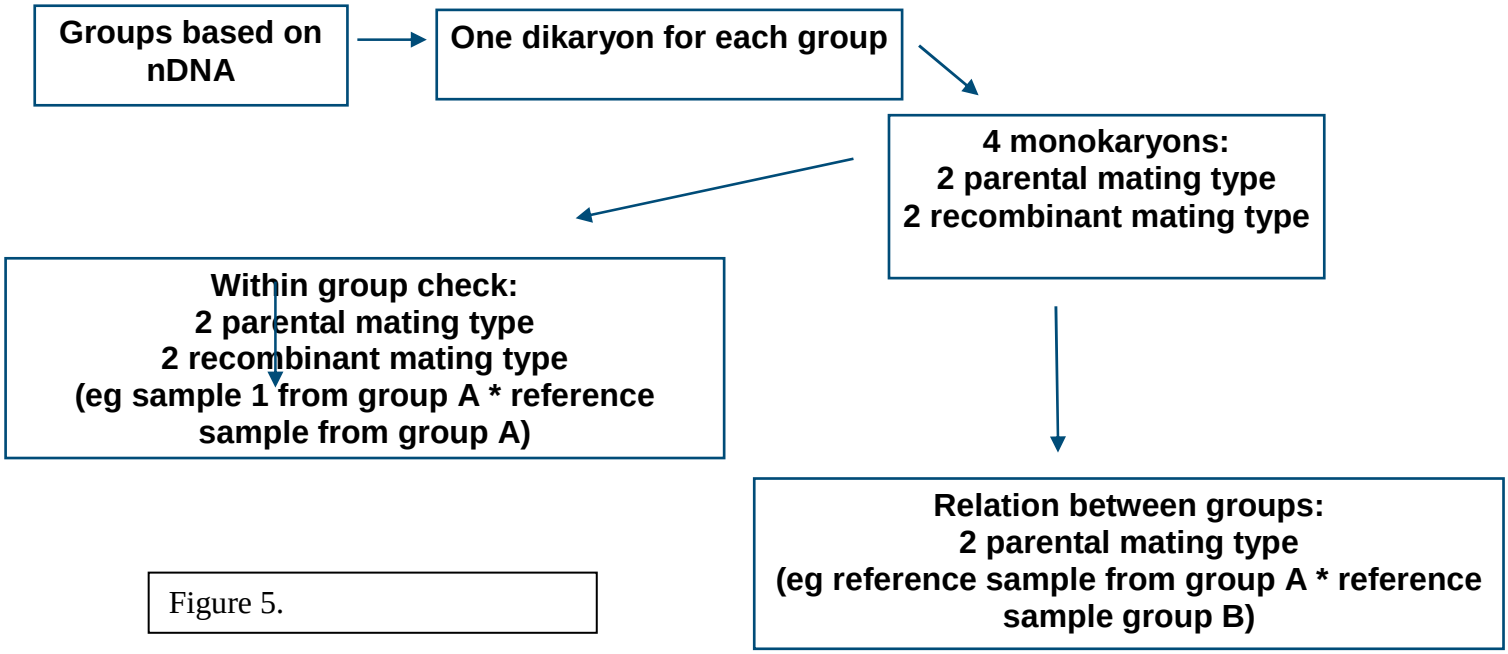


Figure 5.

Although the different genetic groups of mushrooms on the tree log were clustered in groups based on the rpb2 sequences, so far it was impossible to tell whether different groups share the same nuclei. The buller phenomenon was used as a tool to see which nuclei are shared between different groups of dikaryons (Raper 1966 and see Box 1). From each group one sample was used to represent the whole genetic group. From all the representative samples spores were isolated. The mating type of all mycelia was identified by crossing one reference spore with the original parental mycelium in a di-mon mating and by crossing all other spores with the reference spore. The di-mon cross showed which spores are recombinants by producing the flat phenotype in the monokaryon side of the cross. Each of the four mating types was stored for future use. The spores that had equal mating types with one of the parental nuclei were used to reveal the between group similarities. All different spores (2 spores with parental mating type and 2 with recombinant mating type) were used to check whether all dikaryons within one group (within group check) based on RPB2 sequences really have identical nuclei (see figure 5).

Cytotyping

The cytotyping has been done based on mtDNA restriction fragment length polymorphisms (RFLP). Digestions have been performed on total DNA (mtDNA + nDNA). Since only the mtDNA is of interest, GC cutters were used which will discriminate between mtDNA and nDNA based on the GC percentage; the nDNA is rich in G and C (Specht *et al.* 1983) and therefore the nuclear DNA will be cut in very small pieces. See table 2 for a list of restriction enzymes that have been used.

Table 2. List of restriction enzymes

Sequence:	Name:	Frequency (in mtDNA):
C/CGC	AciI	24
C/CNNGG	BsaJI	14
C/CGG	HpaII	17
C/CGG	MspI	17
CC/NGG	ScrFI	25
GC/NGC	FNU4HI	21
GG/CC	HaeIII	14

Digestions were performed following the manufacturers instructions on 0.5 - 1 µg total DNA. Afterwards samples have been electrophoresed in an 0.6% agarose gel in 0.5x TBE (tris-borate-EDTA) buffer at 7V for 16 hours. The gel was stained using GelRed (2.5 µL for 100 µL gel solution). Two blotting techniques were used for visualization (vacuum and capillary blotting). For hybridization we used probes labeled using the Random Dig labeling Kit (Promega) on isolated mitochondrial DNA.

Results

Nuclear Genotyping

Based on polymorphisms in the rpb2 sequences the mushrooms were grouped. From each group one mushroom was selected as a representative mushroom (representative mushrooms have been indicated with an asterisk table 3). The “within group check” showed that it is not possible to fully distinguish between mating types based on the rpb2 sequences and therefore some samples have been excluded from the groups with the reference samples.

Table 3. Group constitution; samples included all have identical nuclei compared to the reference sample indicated with an asterisk.

Tree and group number		Samples included in group	Samples added to group based on RPB2 marker, but excluded on mating type
Tree 30	1	21*	10, 14, 15
	2	4, 5, 8, 9, 11, 12*, 13, 16, 17, 18, 19	25
	3	3*	20
	4	6*	1, 23
	5	2*	24
	6	22*	
Tree 31	1	1*	11, 12
	2	4, 5, 7*, 8, 9, 10	
	3	2, 3, 6*	
Tree 32	1	7*	
	2	9*, 11, 12, 14	8, 10, 15, 16, 22
	3	1, 21*	2, 3, 4, 5, 6, 18, 19, 23, 24
	4	13*, 17	

To see how the groups are positioned on the tree trunks, please refer to the appendix.

In tables 4 to 6 the results of all crosses between the groups have been listed. Those crosses have been performed with the reference samples (indicated with an asterisk in table 3).Under the table of each tree the mating types of the nuclei of each group have been listed. It should be noted that the genotypes of the mycelia on different trees have not been related to each other for instance A1B1 on tree 31 has not been tested with A1B1 on tree 32 and are very likely different.

Table 4. Tree 30. Interaction between isolated monokaryons. d: the cross produced a dikaryotic mycelium, a: the cross produced a mycelium with a flat phenotype, b: the cross produced a mycelium with a barrage, m: the mycelia were not compatible and the mycelia remain monokaryotic.

		30.21		30.12		30.3		30.2		30.6	
		A1B1	A2B2	A2B3	A2B2	A3B2	A2B4	A2B2	A1B5	A4B2	A2B4
30.21	A1B1			d	d	d	d	d	a	d	d
	A2B2			a	m	b	a	m	d	b	a
30.12	A2B3	d	a			d	a	d	m	d	a
	A2B2	d	m			b	a	d	d	b	a
30.3	A3B2	d	b	d	b			b	b	b	d
	A2B4	d	a	a	a			d	d	d	b
30.2	A2B2	d	m	d	d	b	d			d	a
	A1B5	a	d	m	d	b	d			d	d
30.6	A4B2	d	b	d	b	m	d	d	d		
	A2B4	d	a	a	a	d	m	a	d		

Group 30.21: (A1B1) x (A2B2)
Group 30.12: (A2B3) x (A2B2)
Group 30.3: (A3B2) x (A2B4)
Group 30.2: (A2B2) x (A1B5)
Group 30.6: (A4B2) x (A2B4)

Table 5. Tree 31, see table 4 for explanations

		31.1		31.6		31.7	
		A1B1	A2B2	A3B3	A4B4	A5B4	A4B3
31.1	A1B1			d	d	d	d
	A2B2			d	d	d	d
31.6	A3B3	d	d			d	b
	A4B4	d	d			b	a
31.7	A5B4	d	d	d	b		
	A4B3	d	d	b	a		

Group 31.1: (A1B1) x (A2B2)
Group 31.6: (A3B3) x (A4B4)
Group 31.7: (A5B4) x (A4B3)

Table 6. Tree 32. , see table 4 for explanations

		32.7		32.13		32.21		32.9	
		A1B1	A2B2	A3B3	A2B4	A4B5	A5B6	A2B7	A6B8
32.7	A1B1			d	d	d	d	d	d
	A2B2			d	a	d	d	a	d
32.13	A3B3	d	d			d	d	d	d
	A2B4	d	a			d	d	a	d
32.21	A4B5	d	d	d	d			d	d
	A5B6	d	d	d	d			d	d
32.9	A2B7	d	d	d	a	d	d		
	A6B8	d	d	d	d	d	d		

Group 32.7: (A1B1) x (A2B2)
Group 32.13: (A3B3) x (A2B4)
Group 32.21: (A4B5) x (A5B6)
Group 32.9: (A2B7) x (A6B8)

The first two trees (31 and 32) had no conflicting results and the mating types of the nuclei have been resolved. The last tree (tree 30) on the other hand did have some conflicts. Because of the conflicts the most likely genotypes have been displayed. This resulted in one of the dikaryon group (group 30.12) ending up with the mating types (A2B3) x (A2B2), which is not possible.

Cytotyping

RFLPs have been found in the mtDNA. It is questionable whether this technique provides enough resolution to distinguish between different cytotypes (see figure 5.)

A B C D E F G H

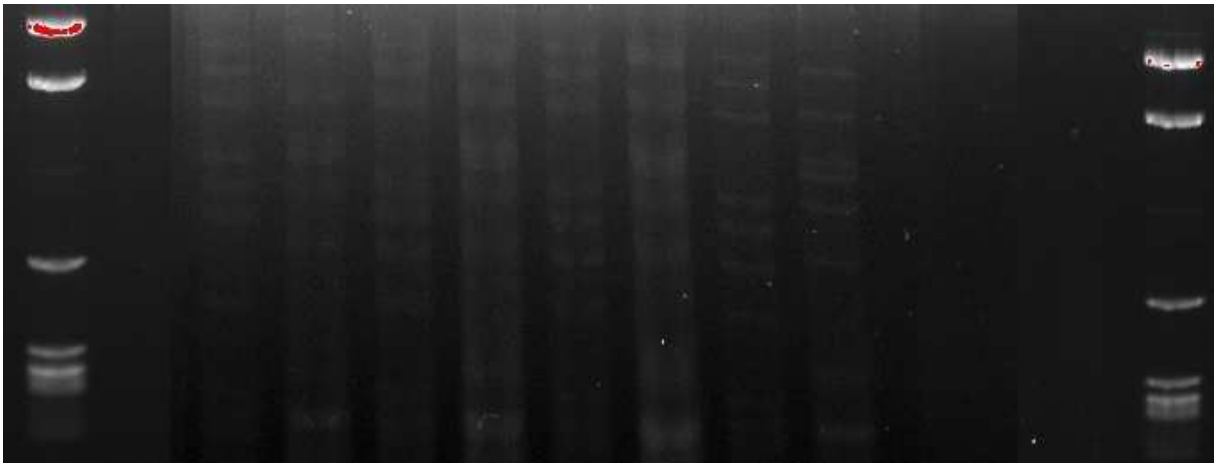


Figure 5. A) sample 32.5 digested with Hae III, B) sample 32.5 digested with Hpa II, C) sample 31.3 digested with Hae III, D) sample 31.3 digested with Hpa II, E) sample 30.1 digested with Hae III, F) sample 30.1 digested with Hpa II, G) sample 31.11 digested with Hae III, H) sample 31.11 digested with Hpa II.

Discussion

This research provides some useful new information with regard to natural nuclear distribution in basidiomycetes. It was found that multiple dikaryons (at least 6 on tree 30) can exist on one tree branch. Since in two cases all the nuclei which were found on a particular branch were unique in genotype the amount of unique nuclei is high which indicates that the buller phenomenon might not occur very frequently.
It is interesting to see that some mating type factors manage to reappear in multiple genotypes in combination with different complementary mating type factors. For instance, in tree 32 mating type factor A2 appears with B2, B4 and B7. Recombination takes place when basidiospores are formed allowing mating type factor A2 to reappear with different B mating type factors. However, since *all* B mating type factors are unique on this tree this type of recombination is unlikely to have taken place. If this is true, mating type factor A2 is present in multiple genotypes on other trees which could have produced the basidiospores which have given rise to the different genotypes containing the mating type factor A2 on this tree. The probability that this has happened can be expected to be low since the chances of multiple A2 mating type nuclei being produced and ending up on the same tree are likely to be very small. Even if the spread of spores is very local, the number of different haplotypes within a local population of *S. commune* is so high that it is unlikely that multiple spores with identical mating type factors will germinate on the same tree (James *et al.* 2001). An alternative explanation may be that recombination occurred during an incompatible buller cross as described by Papazian (1954). Although there is no case of incompatibility in any of the crosses in this research, these results suggest that the phenomenon of recombination during mating might also be induced by other factors. Ellingboe (1964) for instance showed that somatic recombination can also take place.
In this research we hypothesized that distribution of nuclei is realized by three mechanisms. The first one is that a monokaryon gets a nucleus through reciprocal exchange when it comes into contact with another monokaryotic mycelium. This mechanism may act in nature, but there is no evidence from this research due to a lack of data on cytotype distribution.
Tree 30 provides evidence for the second mechanism, fertilization of a monokaryotic mycelium by another dikaryotic mycelium. The nucleus with mating type A2B4 appears in two instances. The accompanying nucleus is different in both cases which excludes reciprocal exchange that would occur in mon-mon mating, as this results in two identical nuclei in both mycelia. Alternatively this particular nucleus may be donated by a basidiospore that is produced by a neighboring fruiting body.
The last mechanism that was hypothesized was that a monokaryotic mycelium acquires a new nucleus as it gets fertilized by a basidiospore. Although this research provides no direct support for this hypothesis, it may be true that this mechanism acts in nature. It is evident that either the first or third mechanism must have taken place in order to get the tree branches populated with *S. commune* in the first place.
Furthermore, we found many different mycelia on each substratum; a prerequisite for mon-mon and di-mon matings. From the 60 samples fruiting bodies 13 different dikaryons were obtained, which is an underestimation, because 24 samples were not analyzed. These 24 are likely to contain at least another 8 different dikaryons.
Since multiple nuclei can populate a single branch, multiple basidiospores must have been involved in the contribution of nuclei. In order for nuclei to be successful they have to be in mycelia which have enough resources to their disposal (usually this is size dependent) to grow mushrooms, only than will the nucleus be able to fulfil its sexual cycle. As a consequence this resource competition leads to mating competition through which nuclei can acquire more mycelia and thus increase the chance to end up in a spore producing mushroom. Although

mating compatibility is regulated by the mating-type genes, it may well be that incompatible matings are overcome by recombination within the dikaryon and dedikaryotization in order to gain more mycelium (Johannesson and Stenlid 2004). If this is true this may indicate that selection happens at the level of nuclei and not on the level of the dikaryon because these mechanisms allow a nucleus in a dikaryon to break up the relation with the other nucleus (see also Johannesson and Stenlid 2004).
Unfortunately, the cytoplasm profiles of the fruiting bodies could not be obtained. Genotypes in combination with cytotypes give more information about how the nuclear distribution in the population arose. This information would clarify distinctions between the three hypothesized scenarios. This research shows that polymorphisms in the mitochondrial genomes do exist, however more research is needed to see whether RFLP methods provide enough resolution to distinguish between mycelia based on cytotype. If RFLP will not work, alternatively sequencing of (part of) the mitochondrial plasmid of all fungal samples might yield this information.

Literature

Aanen, D. K., Kuyper, T. W., Debets, A. J. M. and Hoekstra, R. F., 2004 *The evolution of non-reciprocal nuclear exchange in mushrooms as a consequence of genomic conflict*. Proceedings of the Royal Society of London Series B-Biological Sciences **271:** 1235-1241.

Anderson, J. B., and Kohn, L. M., 2007 Dikaryons, diploids, and evolution, pp. 333-348 in *Sex in Fungi: molecular determination and evolutionary implications*, edited by J. HEITMAN, J. W. KRONSTAD, J. W. TAYLOR and L. A. CASSELTON. ASM Press, Washington D.C.

Buller, A. H. R., 1930 *The biological significance of conjugate nuclei in Coprinus lagopus and other hymenomycetes.* Nature**:** 686-689.

Chen, R. S., and Mcdonald, B. A., 1996 Sexual Reproduction Plays a Major Role in the Genetic Structure of Populations of the Fungus Mycosphaerella graminicola, pp. 1119-1127.

Dons, J. J. M., Devries, O. M. H. and Wessels, J. G. H., 1979 *Characterization of the genome of the basidiomycete Schizophyllum commune*. Biochimica Et Biophysica Acta **563:** 100-112.

Ellingboe, A. H., 1964 *Somatic recombination in dikaryon K of Schizophyllum commune*. Genetics**:** 247–251.

James, T. Y., Moncalvo, J. M., Li, S. and Vilgalys, R., 2001 *Polymorphism at the ribosomal DNA spacers and its relation to breeding structure of the widespread mushroom Schizophyllum commune*. Genetics **157:** 149-161.

Johannesson, H., and Stenlid, J., 2004 *Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete Heterobasidion annosum*. Fungal Genetics and Biology **41:** 563-570.

Kauserud, H., and Schumacher, T., 2003 *Genetic structure of Fennoscandian populations of the threatened wood-decay fungus Fomitopsis rosea (Basidiomycota)*. Mycological Research **107:** 155-163.

Kothe, E., 1999 *Mating types and pheromone recognition in the homobasidiomycete Schizophyllum commune*. Fungal Genetics and Biology **27:** 146-152.

Liu, Y. J. J., Whelen, S. and Benjamin, D. H., 1999 *Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit*. Molecular Biology and Evolution **16:** 1799-1808.

Matheny, P. B., 2005 *Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales)*. Molecular Phylogenetics and Evolution **35:** 1-20.

Papazian, H. P., 1954 *Exchange of incompatibility factors between the nuclei of a dikaryon.* Science**:** 691-693.

Quintanilha, A., 1937 *Contribution à l'étude génétique du phénomène de Buller*. C.R. Acad. Sci.**:** 747.

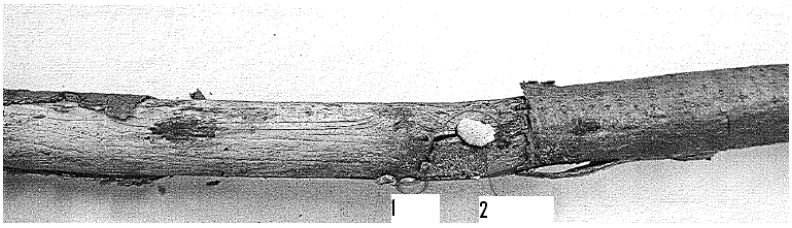
Raper, J. R., 1966 *Genetics of Sexuality in Higher Fungi*. Ronald Press.

Sambrook, J., Fritsch, E. F. and Maniatis, T., 1989 *Molecular cloning : a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

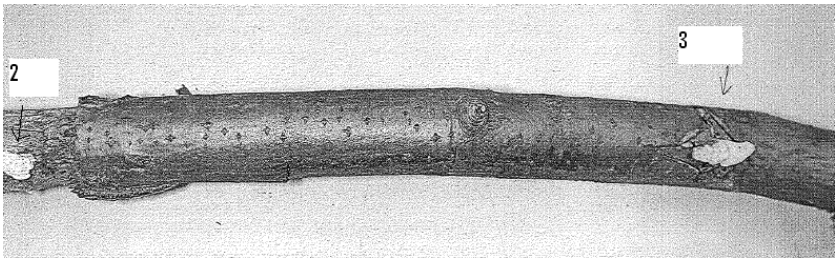
Specht, C. A., Novotny, C. P. and Ullrich, R. C., 1983 *Isolation and characterization of mitochondrial DNA from the basidiomycete Schizophyllum commune*. Experimental Mycology **7:** 336-343.

Appendix

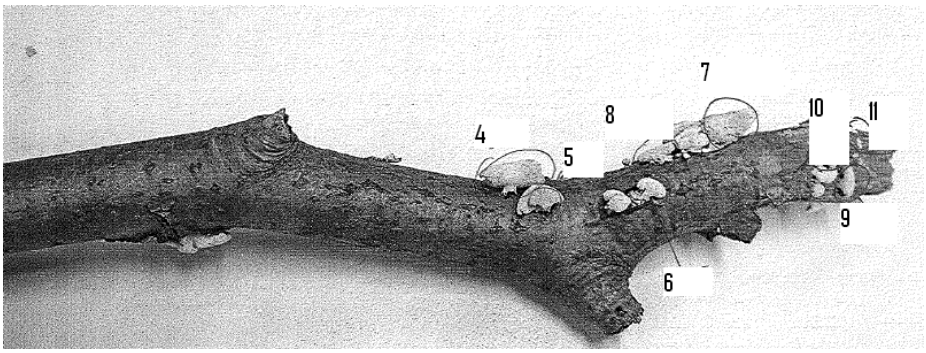
Tree 30



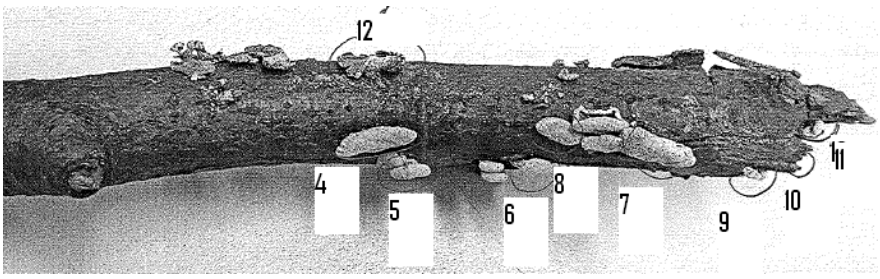
Tree 31 part 1



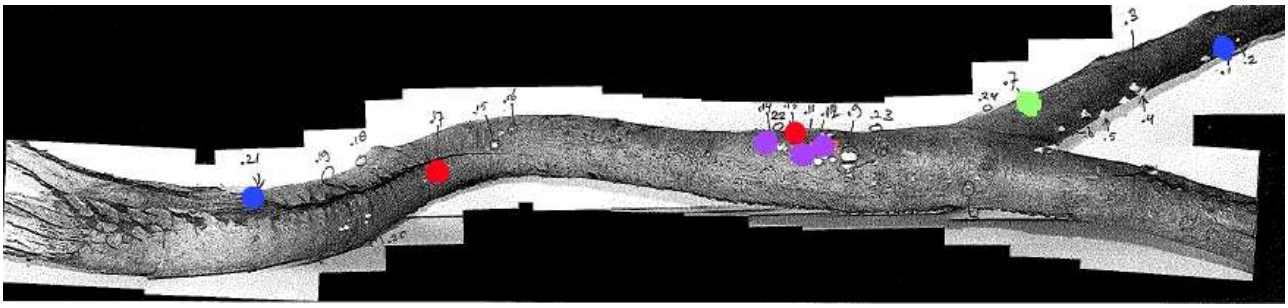
Tree 31 part 2



Tree 31 part 3



Tree 31 part 4



Tree 32