An Assessment of Genetic Variation in *Stereum hirsutum* (Basidiomycota) Based on RAPD Markers

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Abstract

Randomly amplified polymorphic DNA (RAPD) marking with five decamer primers (OPA-01, OPA-04, P36, P46, P49) was applied to 41 samples, which were fruitbody tissue isolates of corticioid wood decay fungus *S. hirsutum* from 18 collection sites in Belarus, situated 0.18–278 km apart. Each primer produced a unique fingerprint for each individual genotype. The samples belong to 35 different genotypes; the identity of RAPD profiles was observed for the samples collected from the same substratum unit. Among 65 reproducible RAPD loci selected for the analysis, there were no strictly monomorphic ones; a single marker had very high frequency (0.94); 29% of markers had the frequencies more than 0.5; the average marker frequency was 0.36. Cluster analysis showed no groups correlated with host, substratum type, or geographical origin, except 3 genotypes from the same collection site, forming a separate cluster. A collection site with the biggest subsample (26% of all studied genotypes) possessed 85% of bands and 100% of them were polymorphic inside this subsample. Average Dice similarity coefficient was almost identical between the genotypes within collection sites (less 0.15 km apart; S_D=0.53) and for the widely separated individuals (more 40 km; S_D=0.52). The molecular data infer highly outcrossing reproduction mode in the studied regional sampling of *S. hirsutum* and the absence of any genetic differentiation.

Key words: Belarus, decamer primers, fingerprints similarity, outcrossing, Russulales

Introduction

Stereum hirsutum (Willd.) Gray is a cosmopolitan corticioid member of the russuloid clade (Binder et al. 2005). It occurs especially often in boreonemoral and nemoral biomes, inhabits mostly Angiospermae, and causes white rot in both fallen wood and in standing trees and shrubs. In the last case, fructifications appear mostly on recently dead trunks and dead still-attached branches. In the same time, the nutritional mode of *S. hirsutum* is not completely studied, and the initial development of its mycelia in living tissue can be more often event than it is usually recorded. In Belarus, this fungus rather often develops on *Quercus robur* in forests, provoking trunk decay in growing trees, and on cultivated *Prunus* spp., provoking trunk and limb die (Yurchenko 2008). The fungus is characterized by great morphological variation and considered on worldwide scale as a species complex (Chamuris 1988).

The aim of our research was to reveal the degree of genetic variation in *S. hirsutum* in a sample from Belarus, representing main hosts, different substratum types, and different geographic position, and to disclose the population structure. Randomly amplified polymorphic DNA marking (RAPD-PCR) was applied by us to evaluate genetic similarities between samples, and to characterize the total genomic variation. The genus *Stereum* and the Stereaceae in total were little embraced by studies into genetic variability: e.g. *S. sanguinolentum* populations in Baltic region were analysed by arbitrary primed PCR with M13 core sequence (Stenlid and Vasiliauskas 1998). No special publications, devoted to population structure in *S. hirsutum*, based on DNA fingerprinting data, were promulgated before.

Material and methods

Forty-one *S. hirsutum* samples were collected in central, eastern and southern parts of Belarus, from 7 administrative districts (Fig. 1), from 9 hosts and 9 substratum types (Table 1). Samples collected within a distance of 150 m have been assigned to the same collection site. The distances between collection localities were from 0.18 km (sites 15 and 16) to 278 km (sites 9 and 18).

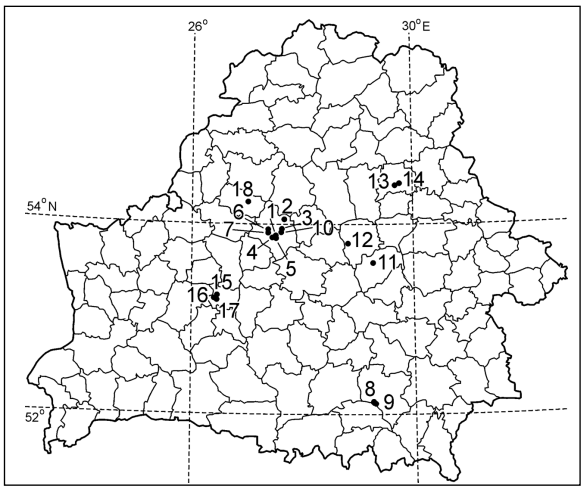

Figure 1. Arrangement of *S. hirsutum* collection sites in Belarus, relatively to the borders of administrative districts. The sites are numbered according to Table 1

Table 1. Data on *S. hirsutum* samples studied

Collection site No. and geographic marker	DNA sample No.* and substratum type**	Host
1 (Minsk, Loshytsa)	**4** (db); **5**, **40** (wb); **6**, **7** (wt); **8** (dt); **9**, **10** (db); **41** (dt); **42** (dt); **43** (dt); **44** (dt)	*Prunus domestica*
2 (Astrashytski Haradok)	**11** (dt)	*Malus domestica*
3 (Minsk, NE part)	**12** (db)	*M. domestica*
4 (Minsk, Loshytsa)	**13** (db)	*M. domestica*
5 (Minsk, Loshytsa)	**14** (wt); **21**, **36–39** (dt); **22** (+b)	*Corylus avellana*
6 (Minsk, W part)	**15** (wt)	*Fraxinus excelsior*
7 (Minsk, W part)	**16** (+t); **17** (+s); **18** (wt)	*Quercus borealis*
8 (Rudnya Harbavitskaya)	**19** (wt)	*Q. robur*
9 (Rudnya Harbavitskaya)	**20** (db)	*Q. robur*
10 (Minsk, Chalyuskintsau Park)	**45** (wt)	*Padus serotina*
11 (Zakutski Dvarok)	**50** (+fb)	*Alnus glutinosa*
12 (Novaya Martsiyanauka)	**51** (wt)	*Quercus robur*
13 (Azyartsy)	**52** (+s)	*Betula pendula*
14 (Mikhailaushchyna)	**53** (+t); **54** (+ft)	*Corylus avellana*
15 (Halautsy)	**55** (+b)	*Quercus robur*
16 (Halautsy)	**56** (+ft)	*Betula pendula*
17 (Halautsy)	**57** (+ft)	*Corylus avellana*
18 (Krasnae)	**58** (wt); **59** (dt); **60** (+b); **61** (+ft)	*C. avellana*

*In the text used with prefix 'Sh'; samples from the same substratum unit are separated by commas. **db – dying branch, dt – dying trunk, wb – wounded living branch, wt – wounded living trunk, +b – dead still-attached branch, +fb – fallen branch, +ft – fallen trunk, +s – dead stump, +t – dead standing trunk

Cultures obtaining and DNA extraction. Isolates were obtained by inoculating washed fruitbody fragments on 2% malt agar with ampicilline 200 units/mL. For biomass accumulation the mycelia were cultivated in flasks containing 13–25 mL of 2% liquid malt for 10 days, at 26ºC in darkness. The total cell DNA was extracted following the protocol described in Ramsfield et al. (1996), with modifications (Yurchenko and Sinyavskaya 2007). Sample DNA concentrations were measured by Ultrospec 3300 pro spectrophotometer (Amersham Biosciences, Sweden) and all samples were diluted by deionized water (milliQ) to the work concentration 10 ng DNA/μL before PCR.

RAPD-PCR protocol. Five decamer primers from OPA series (designed by Operon Technologies Inc., USA, manufactured by PrimeTech, Minsk) and P series (designed in Yu.M. Sivolap Laboratory, Odessa, manufactured by Syntol, Moscow) were randomly selected from the available laboratory stocks (Table 2). Reaction mix for OPA primers, with the final volume 15 μL, included 1.5 μL of the work solution of DNA sample, 1.5 μL of *Taq*-buffer with KCl (Fermentas MBI, Lithuania), 1 μL of primer dissolved to 6 pmol/μL, 1.5 μL of 25mM $MgCl_2$ (Fermentas), 1.5 μL of 2.5mM dNTP mix (Fermentas), 0.25 μL of *Taq*-polymerase (Dialat, Moscow). Reaction mix for primers of P series, with the final volume 20 μL, included 4 μL of the work solution of DNA sample, 2 μL of *Taq*-buffer with KCl, 1 μL of primer diluted to 4 pmol/μL, 2.4 μL of 25mM $MgCl_2$, 1.6 μL of 2.5mM dNTP mix, 0.2 μL of *Taq*-polymerase. Negative controls included the equal volume of milliQ instead of DNA.

The amplifications were performed at GeneAmp PCR System 2700 (Applied Biosystems) thermal cycler. The program for OPA primers included initial denaturation 5 min at 94°C, denaturation 10 s at 94°C, annealing 30 s at 36°C, elongation 1 min at 72°C, the last three stages repeated 35 times, and final elongation 5 min at 72°C. Amplifications with P primers were performed with initial denaturation 2 min 12 s at 94°C, initial annealing 2 min at 39°C, initial elongation 2 min at 72°C, denaturation 40 s at 94°C, annealing 1 min 40 s at 47°C, elongation 1 min 30 s at 72°C, the last three stages repeated 35 times, and final elongation 7 min at 72°C. PCRs for each sample/primer combination were repeated at least twice.

RAPD fragments resolving. PCR products were fractioned in 2% agarose gel stained by EtBr (0.5 μg/mL) at 65 V, at the same gel with standard DNA size markers; profiles were photographed through red filter in UV light by digital camera; fragments were read and compared visually at digital images by the facilities of Adobe Photoshop 5.5 software.

Data analysis. Only the bands repeating in all replicates (reproducible fragments) and that were bright at least in one of 41 samples, were selected for analysis. The bands having the same mobility on the

profiles were admitted as the same marker with two conventional 'allelic' states: present (1) and absent (0). The present state (1) was scored for the data matrix irrespective of the band relative intensity.

Primers resolving power (Rp), as the ability to show genomic differences between samples, was calculated according to Prevost and Wilkinson (1999) formula. Genetic similarity between genotypes was assessed via Dice coefficient: $S_D(i_1, i_2) = 2a/(2a + b + c)$, where a is the number of shared bands, b – number of bands, present in i_1 profile, but absent in i_2, and c – number of bands, present in i_2 profile, but absent in i_1. Mean phenotypic gene diversity (Hp), based on each band presence and absence frequency, was calculated according to the formula in Mariette et al. (2002). Cluster analysis was performed for the combined dataset (binary matrix) for the all loci, and for each primer products separately, by unweighted pair-group method based on the arithmetic average (UPGMA) with Euclidean distance as a genetic similarity measure, by means of Statistica 5.0 software (StatSoft Inc., USA).

Results

Characterization of RAPD markers and resolving individual genotypes. Comparative analysis of the RAPD products has with all primers revealed identical profiles within four sets of samples, originated from single resource unit (trunk of branch): Sh5 and Sh40; Sh6 and Sh7; Sh9 and Sh10 (Fig. 3); Sh21, 36, 37, 38, 39. Based on this identity, we assigned them to the four fungal genets. For the rest 30 isolates, PCR with each of five applied primers produced unique fingerprint for each tested sample, suggesting that each of them represents an individual genotype. As a result, 34 different genotypes were distinguished among 41 studied samples.

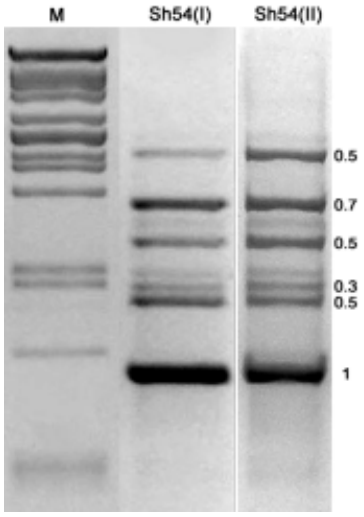

Figure 2. Two repeats of the same RAPD-PCR of Sh54 with primer P36. M: λ-DNA digested with PstI, as molecular size marker

The number of reproducible bands per profile was from 3 (Sh52/P49) and 5 (Sh22/P36; Sh5/P46; Sh41/P49) to 15 (Sh14/P46), 17 (Sh12/OPA-01) and 22 (Sh60/OPA-04). An example of products obtained in two replicates of the same PCR is given in Figure 2. All primers, except of OPA-04, had high resolving power values (Table 2).

Morphologically, all collected fruitbodies, served as the source of isolates, belonged to *S. hirsutum* var. *hirsutum* according to Chamuris (1988) concept, demonstrating moderate variation in fruitbody size and shape, which were conditioned mostly by the degree of fruitbody development and substratum orientation, and some variation in pileus zonation. Genotypic variation occurred to be much higher than the observed morphological variation. Altogether 65 RAPD loci (markers) of assumed molecular identity were selected for analysis (Table 2). No a marker present in all samples was detected. Single marker (OPA-04, 0.87 Kbp) had the very high frequency (0.94). Seven bands (11% of the total number), were present in more than 75% of different genotypes. Nineteen bands (29%) had the frequencies more 0.5. The average frequency of markers was low in the total sampling (0.36), but only two RAPD loci of 65 were unique for a single genotype.

Table 2. Primers and their PCR products characterization

Name	Sequence (5'…3')	Number of scored loci	Approximate size limits for scored fragments, Kbp	Resolving power (Rp)
OPA-01	CAGGCCCTTC	16	0.50–2.03	7.0
OPA-04	AATCGGGCTG	7	0.68–1.10	3.3
P36	CCGAATTCGC	17	0.78–4.40	9.8
P46	GGTTGGGGAG	12	0.72–1.18	10.2
P49	GACAGCCTAC	13	0.75–2.00	9.6
total		**65**		

Genotypic diversity and similarities. When *S. hirsutum* was sampled in the field, the very different spatial density of individuals was observed in collection sites, which was the obstacle to collect equal number of samples from equal plots. Little density was in natural forests non-affected by sufficient human activity. The highest density was in *Prunus domestica* orchard (locality 1), which enabled to collect the biggest subsample there. It was stated, that the diversity of RAPD loci, observed in population on the area more 250 km in extension, was well represented in subpopulations (Table 3). A little number of bands, specific to a collection site, was recorded: single band on site 7 (Sh18), one locus on site 14 (both in Sh 53 and 54), single band on site 18 (Sh60).

To determine possible dependence of genetic similarity from spatial factor, the genetic distances (S_D) between all samples, grouped according to spatial

Table 3. Characteristics of the genetic diversity in two *S. hirsutum* subsamples compared with the total sample

Sample	% of genotypes	No. and % of bands	No. and % of polymorphic bands	Mean phenotypic gene diversity (Hp)
Total	100	65 / 100%	100%	0.328
Collection site 1	26	53 / 82%	100%	0.374
Collection site 18	12	44 / 68%	34 / 77%	0.318

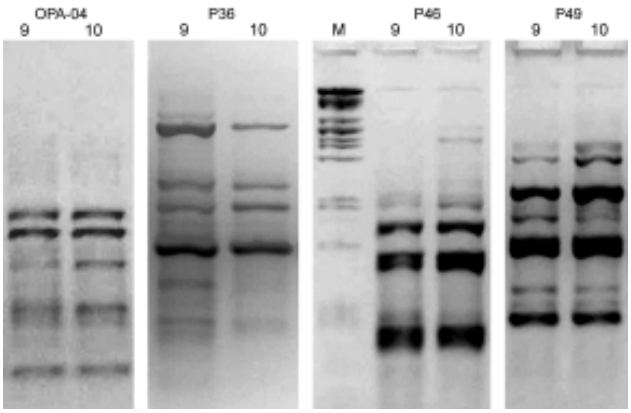

Figure 3. The identity of RAPD profiles, obtained with four primers, for *S. hirsutum* samples 9 and 10, isolated from the basidiomata situated several cm apart on the same living branch of *Prunus domestica*. M: λ-DNA digested with PstI, as molecular size marker. Gels OPA-04, P36, and P49 are in the same scale as P46

scale, were calculated. Average S_D for all pairs, for the individuals within collection sites, and for the individuals, situated more than 40 km apart, appeared to be approximately the same (Table 4). A difference between closely arranged and distant individuals was in the lowest S_D value, which was 0.34 for the first and 0.26 for the second ones. The biggest similarity (S_D=0.84) was between Sh12 and Sh42, arranged on the distance 11 km. The biggest dissimilarity (S_D=0.26) was observed between Sh54 an Sh56, situated 253 km apart.

Table 4. Conventional genetic distance between studied *S. hirsutum* genotypes, evaluated by SD

Pairwise compared genotypes	Arithmetical mean	Range
total sample	0.53	0.26–0.84
in small spatial scale (no more than 150 m between ndividuals)	0.53	0.34–0.76
in large spatial scale (40–278 km between individuals)	0.52	0.26–0.79

To elucidate what kind of genotypes is predominating – significantly similar, moderately similar, or slightly similar – the genetical distance coefficients (S_D) for 561 pairs of the total sample were ranged in 10 classes and a distributional bar graph was built (Fig. 4). It has demonstrated normal distribution with the distinct predominance of moderate similarity between genotypes.

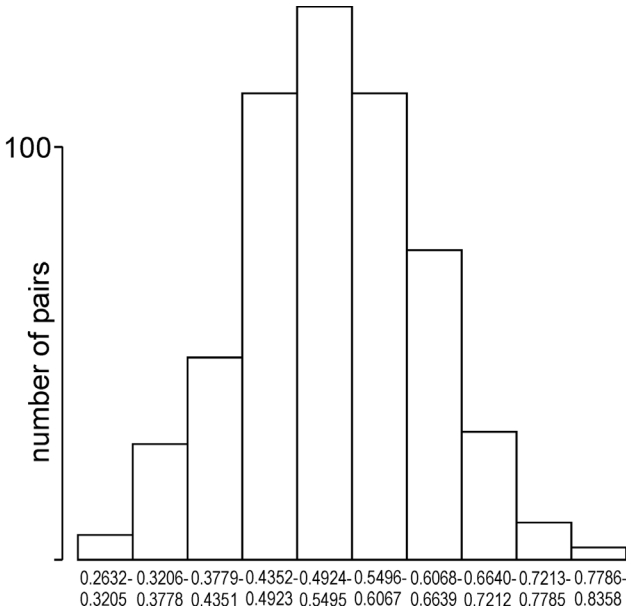

Figure 4. Graph of the distribution of similarity coefficients (SD) between pairwise compared *S. hirsutum* genotypes of the total sample

Cluster analysis. The results of cluster analysis, based on the combined dataset of all markers, are shown on Fig. 5. According to the phenogram and Euclidean distance (dE) values, the similarities are low in general, and no pair of isolates with dE less than 3.3.

Our sample embraced the most frequent hosts for *S. hirsutum* in Belarus: *Corylus avellana*, *Quercus robur*, *Betula pendula*, *Prunus domestica*. No clusters were observed, associated with host taxonomic belonging and substratum type. The single case, when the belonging of the isolates to the same locality was associated with similarity in RAPD markers, is Sh59, 60 and 61, forming a separate cluster. Atthe same time Sh58 from the same locality occurred in the phenogram in a remote position from them. Some individuals situated several meters apart, like Sh53 and Sh54 (the last from fallen trunk originated from the same bush of *Corylus* with Sh53), occurred to be separated considerably on the phenogram. Five main groups (G1–G5) with linkage distance more than 4.6 can be distinguished. The individuals inhabiting site 1 occurred to be dispersed among four large clusters of five (they are absent in G5), or actually they belong to four of five main generalized RAPD patterns.

The cluster analyses involving RAPD markers for each of five primers separately, also did not demonstrate any commonness in respect of geography, host, or substratum type. Distance values (dE) between genotypes were the smallest for OPA-04 dataset, and the biggest for OPA-01 and P36 datasets.

mycelium into ramets or by conidia. The production of sexual spores by selfing (homothallism) also can be considered conventionally in molecular strain typing as clonal reproduction; due to the progeny genomes await to be identical to the parental genome with no recombination (Taylor et al. 1999).

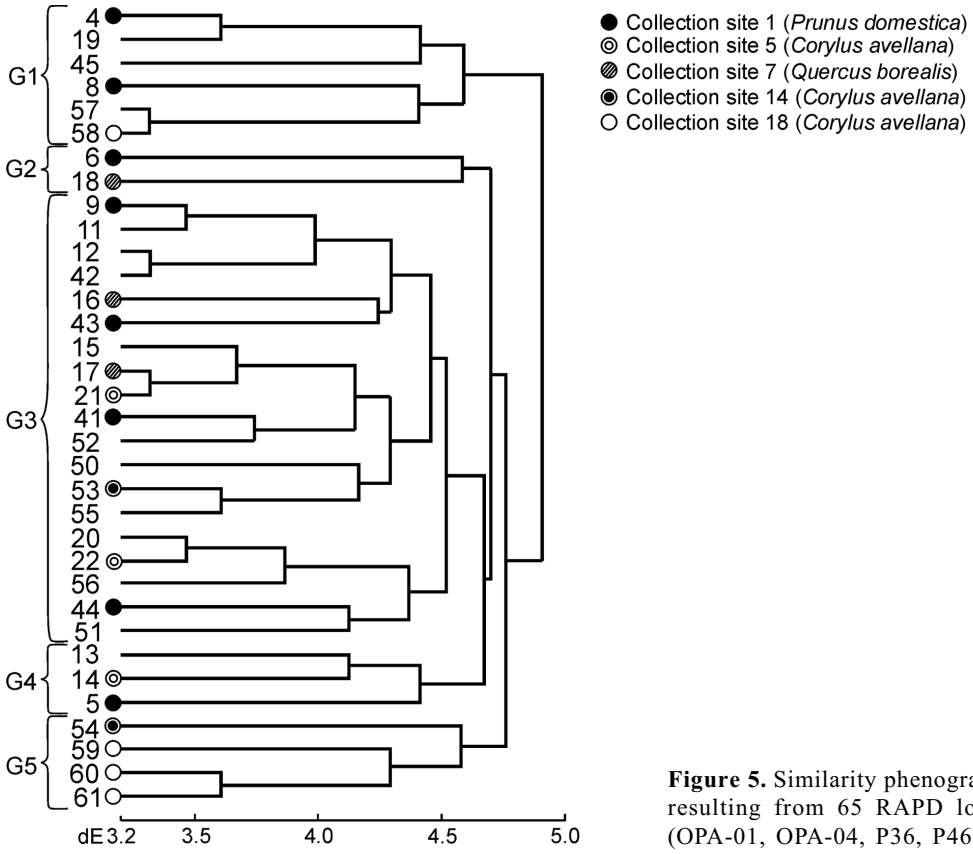

Figure 5. Similarity phenogram for 34 S. hirsutum samples, resulting from 65 RAPD loci produced by five primers (OPA-01, OPA-04, P36, P46, P49)

Discussion and conclusions

The reproduction mode of any fungus is a very important matter for understanding both genetic and phenotype variability in its populations, microevolution events, and for prediction the variation in economically significant traits, like pathogenicity, and in DNA markers, associated with these traits. The most reliable approach to state the mode of reproduction in a given species is to carry out special mating experiments. When the data of biological crossings are not available, an indirect approach is used, when the mode of reproduction is inferred from the structure of fungal populations and distribution of molecular markers. According to a generalized scheme, all fungal species are classified according to their mode of reproduction into recombining (sexual) and clonal (asexual). Clonal fungi in a very broad sense include those propagating by partition of

When population structure is analyzed, there are several main features to distinguish non-recombining fungi from recombining ones. Non-recombining (clonal in broad sense) fungi have (i) presence of overrepresented genotypes, (ii) high association among alleles at different loci, and (iii) the tendency to genetic differentiation among populations. The analysis of total sample in clonal fungus shows the prevalence of highly similar and highly dissimilar genotypes, which become visible via the distributional bar graph for similarity coefficients. Concerning the association between loci, recombining fungi, contrary to clonal ones, have the alleles in many different combinations (Tibayrenc et al. 1991, Taylor et al. 1999).

Among wood-decay representatives of the natural russuloid clade (Russulales) to which *S. hirsutum* belongs, the studies of population genetics, based on DNA fingerprinting methods, were carried out in a small

number of species and genera, viz. *Stereum sanguinolentum*, *Amylostereum*, *Hericium*, *Heterobasidion*, and *Peniophora*. The species from these genera display different reproduction manners.

It was stated that *S. sanguinolentum* is generally non-outcrossing, with strong inbreeding within vegetative compatibility groups (VCGs), which is compatible with DNA markers data. Each VCG in this species had a discrete banding pattern and the similarities in fingerprint profiles within VCGs decreased with increasing geographic distance that indicates a tendency to geographic differentiation (Stenlid and Vasiliauskas 1998). *Amylostereum areolatum* dissiminates mostly by asexual propagules, which leads to clonal structure of its populations and identical DNA banding patterns within such clones. On the contrary, *A. chailletii* propagates mostly by airborne basidiospores produced by outcrossing, and DNA markers showed low genetic differentiation for this species (Vasiliauskas et al. 1998). Zhao and Luo (1999) studied a laboratory population of single-spore isolates from single fruitbody of outcrossing species *Hericium erinaceus* by RAPD-PCR, and revealed the high variation: similarity coefficients between isolates were in the range 0.39–1.0. *Heterobasidion annosum* is highly outcrossing species; it was stated, that most of its genotypes from the same locality had different mating alleles, and thus originated from unrelated basidiospores, which was confirmed by DNA fingerprinting (Garbelotto et al. 1999). Large-scale geographic differentiation was concluded from RAPD data within *Heterobasidion* intersterility groups, equal to biological species (Fabritius and Karjalainen 1993, Garbelotto et al. 1993, Karjalainen 1996), and the presence of population-specific bands was noted (La Porta et al. 1997b). Besides, different level of genetic variation was noted within these biological species. In some regions, this variation is low, e.g. high uniformity in RAPD patterns was stated for the F intersterility group in northern Italy according to La Porta et al. (1997a) and throughout Italy according to Goggioli et al. (1998). La Porta et al. (1997a) stated, that F isolates, originated from the same locality, usually grouped in the same cluster according to RAPD data. In outcrossing species *Peniophora incarnata* (Yurchenko and Sinyavskaya 2008), the RAPD patterns were highly dissimilar within study plots. In outcrossing species *P. quercina,* RAPD marking provided some support for supposed differentiation on a large geographical scale (see Hallenberg 1995: 98).

The question about *S. hirsutum* reproduction mode has a long history. Since clamp-connections occur in this species on the mycelia of single basidiospore origin, *S. hirsutum* was treated as presumably homothallic (Kniep 1920), or homothallic (Boidin 1958). Our observations on *S. hirsutum* monospore mycelia showed, that they have mostly simple septa, but also scattered solitary clamps and verticillate clamps. Coates et al. (1981) experimentally demonstrated that *S. hisutum* populations on British Isles do consist of outcrossing individuals. However, it is now known, that on a global scale this species consists of both outcrossing and non-outcrossing populations (Ainsworth and Rayner 1989; Ainsworth et al. 1990). Coates et al. (1981) provided evidence, that basidiospores of *S. hirsutum* from homokaryotic fruitbody might represent identical genotypes, spread of which is likely to result in genetic homogeneity within a population. Such principle was also confirmed by experiment on spore progeny of homothallic *Agaricus*, where homogeneity in RAPD markers was observed (Calvo-Bado et al. 2001).

The statistical measures, applied by us, have demonstrated a very high level of genetic variation and no features of genetic differentiation in the studied sample of *S. hirsutum*. Firstly, the RAPD patterns were quite dissimilar within collection sites. Then, the average similarity between genotypes was low (0.53) and there was no any significant difference in this parameter for the genotypes within collection sites and between distant collection sites; highly similar genotypes and highly dissimilar ones were not numerous; the present-alleles (state '1') were combined in an arbitrary manner; only several bands were specific for certain collection sites. Taking into account the molecular data on other wood-inhabiting Russulales, and the features in molecular markers distribution, which are assumed for homothallic or clonal fungi, as discussed above, our research showed no evidences of non-outcrossing reproduction manner in the studied sample of *S. hirsutum* from Belarus.

Isolates having identical RAPD patterns, obtained with each of tested primers, are considered by us as clones of the same genet. The occupation of a single trunk by a sole genotype is still not an explained phenomenon, taking into account that such substratum unit can have several infection gates (wound areas) for colonizing by large numbers of genetically diverse (outcrossing) basidiospores. Nevertheless, it should be kept in mind that genetic identity based on RAPD loci can be accepted with very high possibility, but not absolutely, since PCRs with even several decamer primers produce the set of fragments, the length of which embraces only a little part of the total genome. For example, total length of 22 fragments in OPA-04 profile of Sh60 was about 28.7 Kbp, whereas haploid genome sizes known in Basidiomycota representatives are from 16 to 366 Mbp (Kullman et al. 2005). Thus, it is more correct to say about the same *RAPD-genotype*

for samples with identical profiles, if other additional methods for testing identity were not applied. One of such approaches is widely accepted somatic incompatibility test *in vitro* (Coates et al. 1981), but in some cases, the incompatibility reaction is so weak that it is hardly distinguishable from free intermixing of clone mycelia.

The obtained data provide the foundation for intense recombination in *S. hirsitum*. Due to the absence of natural boundaries in the study area, free interbreeding and the belonging of the all tested individuals to the same large population is supposed. Besides, the cluster analysis for *S. hirsutum* demonstrated so-called *unstructured* variation with no correlation of RAPD patterns with host, substratum state, and in almost all cases, with geographic origin.

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References

Ainsworth, A.M. and Rayner, A.D.M. 1989. Hyphal and mycelial responses associated with genetic exchange within and between species of the basidiomycete genus *Stereum*. *Journal of General Microbiology* 135: 1643-1659.

Ainsworth, A.M., Rayner, A.D.M., Broxholme, S.J. and Beeching, J.R. 1990. Occurrence of unilateral genetic transfer and genomic replacement between strains of *Stereum hirsutum* from non-outcrossing and outcrossing populations. *The New Phytologist* 115: 119-128.

Binder, M., Hibbett, D.S., Larsson. K.-H., Larsson, E., Langer, E. and Langer, G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). *Systematics and Biodiversity* 3: 113-157.

Boidin, J. 1958. Hétérobasidiomycčtes saprophytes et Homobasidiomycčtes résupinés: V. Essai sur le genre *Stereum* Pers. ex S.F. Gray. *Revue de Mycologie* (Paris) 23: 318-346.

Calvo-Bado, L.A., Challen, M.P., Thurston, C.F. and Elliott, T.J. 2001. RAPD characterization of heterogeneity in spore progenies and sexuality in the genus *Agaricus*. *Mycological Research* 105: 370-376.

Chamuris, G.P. 1988. The non-stipitate stereoid fungi in the northeastern United States and adjacent Canada [Mycologia Memoir 14], 247 pp. Berlin, Stuttgart: J. Cramer.

Coates, D., Rayner, A.D.M. and Todd, N.K. 1981. Mating behaviour, mycelial antagonism and the establishment of individuals in *Stereum hirsutum*. *Transaction of the British Mycological Society* 76: 41-51.

Fabritius, A.-L. and Karjalainen, R. 1993. Variation in *Heterobasidion annosum* detected by random amplified polymorphic DNAs. *European Journal of Forest Pathology* 23(4): 193-255.

Garbelotto, M., Bruns, T.D., Cobb, F.W. and Otrosina, W.J. 1993. Differentiation of intersterility groups and geographic provenances among isolates of *Heterobasidion annosum* detected by random amplified polymorphic DNA assays. *Canadian Journal of Botany* 71(4): 565-569.

Garbelotto, M., Cobb, F.W., Bruns, T.D., Otrosina, W.J., Popenuck, T. and Slaughter, G. 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. *Phytopathology* 89: 546-554.

Goggioli, V., Capretti, P., Hamelin, R.C. and Vendramin, G.G. 1998. Isozyme and RAPD polymorphisms in *Heterobasidion annosum* in Italy. *European Journal of Forest Pathology* 28(1): 1-88.

Hallenberg, N. 1995. Dispersal abilities and distributional patterns in Aphyllophorales, with emphasis on corticioid fungi. *Symbolae Botanicae Upsaliensis* 30(3): 95-100.

Karjalainen, R. 1996. Genetic relatedness among strains of *Heterobasidion annosum* as detected by random amplified polymorphic DNA markers. *Journal of Phytopathology* 144(7-8): 399-404.

Kniep, H. 1920. Über morphologische und physiologische Geschlechtsdifferenzierung (Untersuchungen an Basidiomyzeten). *Verhandelingen der Physikalisch-medizinischen Gesellschaft* (Würzburg) 46: 1-18.

Kullman, B., Tamm, H. and Kullman, K. 2005. Fungal genome size database. Retrieved at <http://www.zbi.ee/fungal-genomesize>.

La Porta, N., Capretti, P., Kammiovirta, K., Karjalainen, R. and Korhonen, K. 1997a. Geographical cline of DNA variation within the F intersterility group of *Heterobasidion annosum* in Italy. *Plant Pathology* 46(5): 773-784.

La Porta, N., Capretti, P., Korhonen, K., Kammiovirta, K. and Karjalainen, R. 1997b. The relatedness of the Italian F intersterility group of *Heterobasidion annosum* with the S group, as revealed by RAPD assay. *Mycological Research* 101: 1065-1072.

Mariette, S., Le Corre, V., Austerlitz, F. and Kremer, A. 2002. Sampling within the genome for measuring within-population diversity: trade-offs between markers. *Molecular Ecology* 11: 1145-1156.

Prevost, A. and Wilkinson, M.J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* 98: 107-112.

Ramsfield, T.D., Becker, E.M., Rathlef, S.M., Tang, Y., Vrain, T.C., Shamoun, S.F. and Hintz, W.E. 1996. Geographic variation of *Chondrostereum purpureum* detected by polymorphism in the ribosomal DNA. *Canadian Journal of Botany* 74: 1919-1929.

Stenlid, J. and Vasiliauskas, R. 1998. Genetic diversity within and among vegetative compatibility groups of *Stereum sanguinolentum* determined by arbitrary primed PCR. *Molecular Ecology* 7(10): 1265-1274.

Taylor, J.W., Geiser, D.M., Burt, A. and Koufopanou, V. 1999. The evolutionary biology and population genetics underlying fungal strain typing. *Clinical Microbiology Reviews* 12(1): 126-146.

Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Breniere, S.F., Darde, M. and Ayala, F.J. 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. *Proceedings of the National Academy of Sciences of the USA* 88: 5129-5133.

Vasiliauskas, R., Stenlid, J. and Thomsen, I.M. 1998. Clonality and genetic variation in *Amylostereum areolatum* and *A. chailletii* from Nothern Europe. *The New Phytologist* 139: 751-758.

Yurchenko, E. 2008. Corticioid fungi (Basidiomycota) on living woody plants in Belarus: species inventory and host colonization strategies. *Bothanica Lithuanica* 14(3): 177-189.

Yurchenko, E.O. and Sinyavskaya, M.G. 2007. The basics of fungal DNA molecular marking: A laboratory manual. Pravo i ekonomika, Minsk, 101 p. (in Russian with English Contents)

Yurchenko, E.O. and Sinyavskaya, M.G. 2008. A preliminary assessment of genetic variability in *Peniophora incarnata* (Basidiomycetes) based on RAPD markers. *Botanika* (Minsk) 35: 132-143. (in Russian with English summary)

Zhao, W.-H. and Luo, X.-C. 1999. Studies on polarity of the *Hericium erinaceus* and RAPD analysis of its monokaryons. In: Broderick, A. and Nair, T. (eds), *Proceedings of the 3rd International Ńonference on Mushroom Biology and Mushroom Products*. Sydney: University of Western Sydney.

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ОЦЕНКА ГЕНЕТИЧЕСКОЙ ВАРИАЦИИ У *STEREUM HIRSUTUM* (BASIDIOMYCOTA) ПО RAPD-МАРКЕРАМ

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Резюме

Маркирование тотальной геномной ДНК методом неспецифически амплифицированных полиморфных фрагментов (RAPD-PCR) с использованием пяти праймеров-декамеров (OPA-01, OPA-04, P36, P46, P49) было проведено для 41 образца кортициоидного дереворазрушающего гриба *S. hirsutum*, собранных в 18 локалитетах Беларуси, расположенных на расстоянии от 0.18 до 278 км друг от друга. Изоляты были полученные из гиф плодовых тел. Каждый из праймеров продуцировал уникальный набор фрагментов (RAPD-профиль) для каждого индивидуального генотипа. Была установлена принадлежность изученных образцов к 35 различным генотипам; идентичность RAPD-профилей отмечена для образцов, собранных с общего ствола или ветви. Среди 65 воспроизводимых RAPD-маркеров, отобранных для анализа, мономорфные локусы отсутствовали; только 29% маркеров встречались с частотой более 0.5; средняя частота маркеров составляла 0.36. Наиболее крупная из изученных субпопуляций (26% всех генотипов) включала 85% маркеров, все из которых были полиморфны. Кластерный анализ сходства по изучаемым маркерам не выявил групп образцов, объединенных растением-хозяином, типом субстрата и географией, за исключением 3 образцов из общего локалитета, образовавших самостоятельный кластер. Средний коэффициент сходства Дайса по RAPD-локусам для пар близко расположенных индивидов (до 150 м; S_D=0.53) оказался почти идентичен таковому для индивидов, находящихся на расстоянии 40–278 км (S_D=0.52). Молекулярные данные свидетельствуют о гетероталлизме, значительной роли рекомбинации и отсутствии генетической дифференциации в изученной региональной выборке *S. hirsutum.*

Ключевые слова: Беларусь, гетероталлизм, ДНК-фингерпринт, праймеры-декамеры, Russulales