Study of duplicated genes in *Schizophyllum commune*

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Introduction

Gene duplication is one of the main evolutionary mechanisms. The occurrence of an additional copy of a gene weakens selection, allowing one of the copies to change. This could lead to emergence of a new gene function, which makes gene duplications, specifically the most recent, an interesting object for investigation.

To study this, the knowledge of polymorphisms - genome variations in certain individuals – is required. To have enough data for analyses, It is crucial to have the individual genomes differing in vast amount of nucleotides in different positions. This means, that the genome of those species should be polymorphic. The genome of a basidiomycete *Schizophyllum commune* is one of these. As an object of our research we took 13 individuals of these species, with preliminary genome assemblies available.

Aims

- To verify the quality of assembled genomes that belong to the *Schizophyllum commune* family in the regions of predicted duplications
- To study the evolution of recently duplicated genes.

Methods

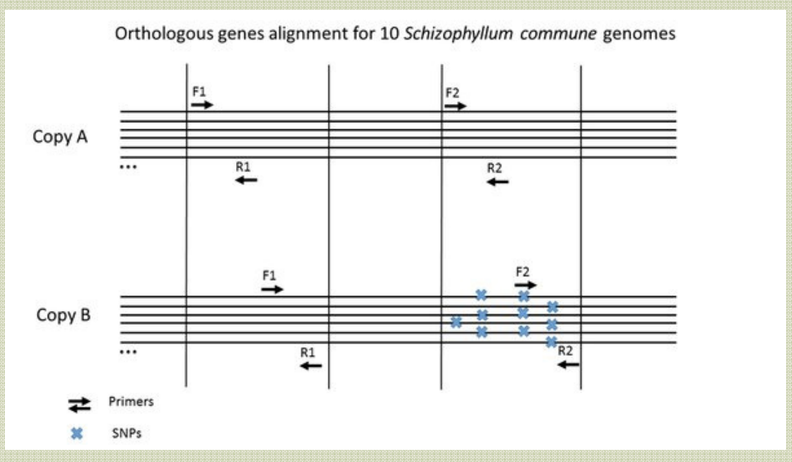
Experimental:

•DNA Extraction (using CTAB)
•PCR (HS Taq DNA polymerase, Evrogen)
•qPCR (Sybr Green I HS mix)
•Measurements of the DNA concentration with Nanodrop and Qubit
•Agarose gel electrophoresis (0,8%-2% of agarose, Sybr safe)
•Purification of PCR products (from the PCR mixes and agarose gels)

Bioinformatical:

•Computations were performed on a remote server ma.fbb.msu.ru
•Reads quality analysis with FastQC
• Exclusion of low quality reads' fragments using
•Alignment of processed reads on the genome using Bowtie2
•Converting ASCII to binary files using Samtools utility
•Alignment visualisation in IGV software

Results

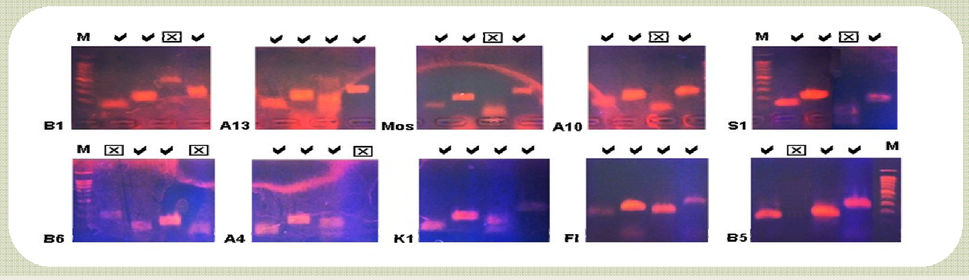


Design of primers for qPCR analysis

DNA extraction and qPCR analysis

DNA was extracted from 10 mushrooms of *Schizophyllum commune* species.
The concentrations of the obtained DNA varied from 30 to 700 ng/µl.

Four fragments of the two duplicated gene copies were analysed by qPCR.
For several genomes including K1, S1, B6 and A10 the duplication was confirmed

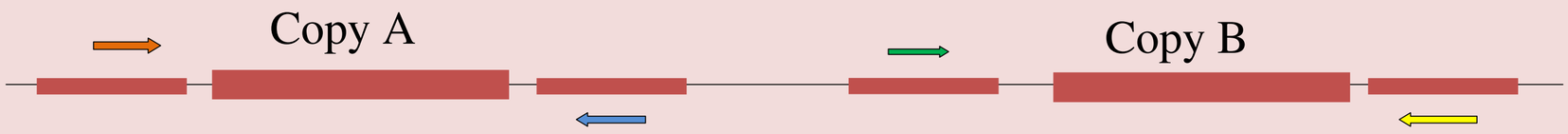


	A		B	
A4	25,0	26,2	27,3	31,0
A10	27,3	25,2	25,9	23,8
A13	26,9	26,7	27,5	27,3
B1	27,1	26,8	29,8	26,6
B5	26,7	-	26,3	28,2
B6	26,1	24,8	24,5	27,9
F1	30,8	28,0	28,3	30,0
Mos	30,4	28,1	25,8	28,2
K1	23,8	23,6	22,9	26,7
S1	23,8	23,9	23,1	24,5

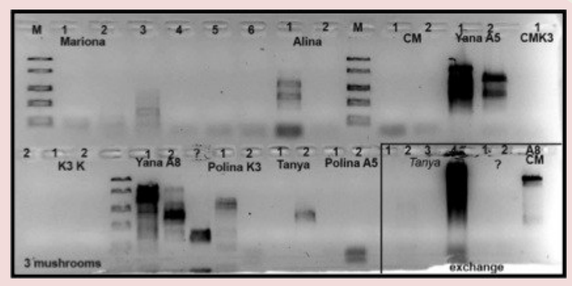
Number of qPCR cycles at which the products were detected (C_T)

Amplification of individual genes

Each copy of studied genes was amplified independently with the specially designed primer pair.



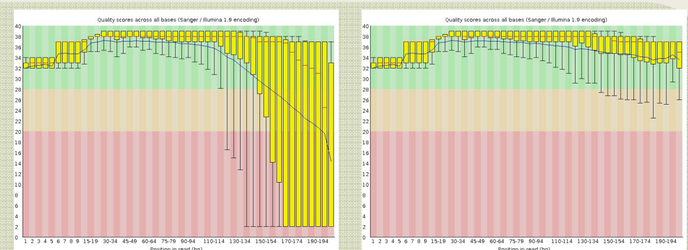
PCR products were then analyzed by agarose gel electrophoresis.
In most cases, the expected products of PCR had not been detected. The obtained products for each genome were put into 2 groups so that for all of the individuals, gene's copy A was separated from copy B. This approach allows to track the polymorphisms of each copy.
The samples then were purified and sequenced on Illumina MiSeq using 200 nt reading kit.



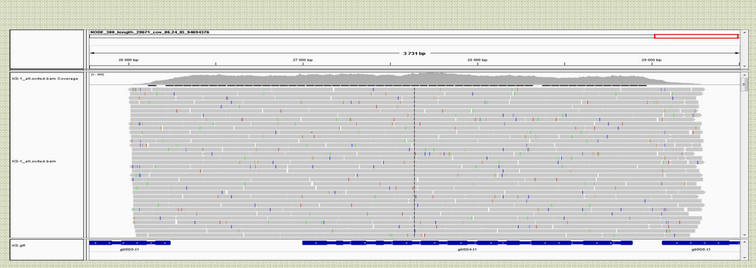
Electrophoresis of PCR products in agarose gel

Analysis of sequencing data

The powerful server is needed to process the large amount of data obtained from sequencing. Thus, we connected to ma.fbb.msu.ru using such utilities as FAR and PuTTY.
Raw data (reads, obtained after the sequencing) usually contain nucleotides of low quality. So, the first step was to delete them.



Raw and trimmed reads quality



Alignment of reads to a particular region in the genome K3

The processed reads were aligned on the genomes of corresponding individuals in order to check whether the correct genomic regions had been amplified.
It turned out, that only small fraction of reads (less than 5 per cent) matched the expected genes.

Conclusions

- After thorough analysis of *Schizophyllum commune* genomes, we came to the conclusion that available whole- genome versions are not suitable for studying duplications on this type of mushroom.
- However, the presence of duplicated genes in *Schizophyllum commune* was confirmed by real-time quantitative PCR.

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