The PaLTEE: *Podospora anserina* Long-Term Evolution Experiment

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The *Podospora anserina* long-term evolution experiment (PaLTEE) is the only running filamentous fungus study [5], which is still going on. Laboratory evolution of microorganisms, informally named “evolution in a flask”, has become a routine task due to the widespread adoption of high throughput DNA sequencing methodology [1]. It allows to detect changes in the genomes of evolved lines by comparison with the original founder strain. However, long-term projects are still few as they are very labor-consuming [3, 4, 6]. So each well-chosen model system contributes to the development of evolutionary biology.

Our research team is tracing the evolutionary dynamics of eight haploid experimental populations of the model ascomycete fungus *P. anserina*. The continuous mycelium growth is maintained in a liquid nutrient medium by the method of synchronous serial passages from flask to flask. The experiment was started in 2012 at Department of Mycology and Phycology, Faculty of Biology, Lomonosov Moscow State University [5]. In current report, we present the results of genome-wide analysis of all experimental lines 8 years after the start of the PaLTEE, which corresponds to 532 passages.

Sequencing reads were obtained using Illumina platform in paired-end sequencing mode. Read pairs were mapped on published *P. anserina* reference genome [2] (NCBI GenBank assembly accession GCA_000226545.1). For reliable detection of fixed *de novo* single nucleotide polymorphisms (SNPs) fairly strict criteria were used. The substitution was not taken into account if at least one of the following conditions was realized: 1) coverage of at least one sample was less than 10 reads; 2) the substitution was present in the genome of the founder line; 3) alternative allele was found at low frequency (<85%) in at least one of the samples. We randomly chose 40 filtered SNPs and performed manual curation of read alignments near variant position in series of samples. Similar algorithm was used to determine short insertion-deletion mutations (indels). But for the indel filtering we examined the region (–10; +10) base pairs around the detected indel coordinate.

The analysis carried out detected 312 SNPs and 39 indels in total. Comparison with our earlier data [5] shows a clear trend towards a linear increase in the number of SNPs depending on the number of passages made. The only one line became an exception: it demonstrated a much higher mutation rate compared to the other evolved populations. This dynamic type may indicate the fixation of the mutator allele.

Among 312 SNPs, 153 were fixed in the coding regions of *P. anserina* genome. Considering the fact that the coding sequences together make up about 47% of the genome examined, our data indicate an almost equal probability of fixing point substitutions in coding and non-coding regions. Just 38 synonymous mutations (also called to a first approximation “silent” mutations) were found among 153 gene substitutions. The evident predominance of significant substitutions suggests that at least some of them may be adaptive under experimental conditions. In other words, they did not arise by chance. Among them, 42 were classified as nonsense mutations (resulting in a stop codon) and 73 as missense mutations (resulting in an amino acid non-synonymous change in the corresponding protein). Slightly more than half of indels identified (21 out of 39) are also localized in coding regions. Most of them (17 out of 21) are frameshift mutations (leading to a shift in the reading frame), which means a probable loss of function of the protein product. The simple empirical rule may be established: once fixed, the mutation persists over time. It works for almost all mutations observed in the PaLTEE.

Parallelisms have been identified in fixing mutations in the same genes of independent *P. anserina* populations. Eight protein-coding sequences evolved in 2 or more lines. With our substitution filtering method, the most outstanding result of fixing new alleles was 6 out of 8 experimental lines. Moreover, such a high substitution number was achieved by 2 different genes. One of them is responsible for coding putative guanine nucleotide-binding protein alpha-1 subunit; another one encodes putative protein with unknown function. Furthermore, we report here for the first time that we discovered parallelisms at the level of paralogs. This category includes 16 putative protein-coding *P. anserina* genes, which form 5 separate groups of homologues. And the homologues within each group are fairly close to each other. According to bioinformatics prediction, the putative protein products of the paralogs described perform exactly the same or very similar biological functions.

Parallel evolution at the level of protein functions were also found in the *P. anserina* model system. The putative products of a number of genes involved in the same biological processes have undergone changes. We emphasize that genes of this type presumably are not homologues to each other. Attention is drawn to a significant proportion of substitutions in putative transcription factors. Almost all the mutations of transcription factors genes potentially affect their product function. Many of the factors reported contain annotated DNA-binding domain. Focusing on the regulation of RNA synthesis may indicate that the selection occurred during the PaLTEE is aimed at optimization a wide range of cellular processes. Indeed, *P. anserina* under study is forced to adapt to a very unusual conditions. Specifically, the fungus lives in a closed environment within a liquid nutrient medium, which is constantly subjected to intensive aeration. The fungi do not encounter anything like this in nature.

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