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WATER AND AIR — SHAPERS OF BIRDS MTDNA EVOLUTION*Y. Gusarov¹, A. Mikhailova¹, B. Efimenko¹, K. Gunbin¹, A. Bushuev², V. Burskaya³, K. Popadin¹¹*Immanuel Kant Baltic Federal University, Kaliningrad*²*Lomonosov Moscow State University*³*University of Antwerp, Belgium*

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Abstract

In mitochondrial DNA (further mtDNA) A>G substitution in protein-coding genes on a heavy strand is the main result of chemical damage (for example, spontaneous deamination). Some vertebrates, like mammals [1] and fishes [2] show strong correlations between mtDNA intense mutagenesis and life-history traits. Here we are trying to discover these connections in birds.

Mitochondria is the main energy producer of the cell with its own DNA molecule. mtDNA is vulnerable to different internal mutagenic factors during replication. Due to replication unevenness, some protein-coding genes mutate more than others [3]. Ones close to the origin of replication (COX1, COX2) spend less time in a single-stranded condition and thus, unlike genes further from origin (CytB), accumulate less mutations. Result of this mutagenic process is A>G substitution on the heavy strand. Recently, different connections between mtDNA mutagenesis speed and life-history traits were discovered for some groups of animals. Amount of mutations in short-living mammals is much lower compared to long-living ones [1]. Fishes, inhabiting warm waters, accumulate more mutations than ones living in cold waters [2]. With this knowledge we decided to discover such connections in birds.

Firstly, we created a database for 766 bird species by using RefSeqs mtDNA data. For each birds we calculated our metric $GhAhSkew = \frac{G - A}{G + A}$, where G shows the amount of guanine in four-fold neutral positions and A shows the amount

of adenine in four-fold neutral positions. This metric shows nucleotide shift due to A>G mutation. We also gathered the most amount of ecological data by using AVONET and Birds of the World databases [4, 5]. To investigate our problem, we used methods like t-test, U-test, linear models, PGLS and reconstruction of ancestral states.

With the help of basic statistical methods, we discovered first interesting results. Birds mtDNA protein-coding genes have a gradient of GhAhSkew, same as it was shown in mammals [1]. COX1 and COX2 genes have low GhAhSkew value, CytB has high one. With this result we decided to compare these two groups of animals. We found that birds GhAhSkew value in most genes is higher compared to mammals. The maximum difference was 1.5x times. We made a conclusion that birds' mutagenesis rate was much higher than in mammals. To find out what was behind such a high mutagenesis rate, we started to compare our metric with different birds' life-history traits. We discovered one metric which lowers mtDNA mutagenesis — losing ability to fly, and several which increase it — getting ability to dive and migration type. To our surprise, there was no connection between mutation rate and many simple metrics, like body mass. Then we checked if our discoveries have any phylogenetic value. We checked only two traits — getting ability to dive and losing ability to fly due to the data limitations. For both metrics PGLS showed p-value < 0,05 and Pagel's lambda value $\lambda > 0.9$. These results prove that these two factors are the main reason behind birds' mtDNA nucleotide shift due to A>G mutation. So we decided to find out when the mutagenesis happened and if it continues right now with the same speed. For that we reconstructed mtDNA mutational spectrum for our birds and compared it with our phenotypes. PGLS showed no correlation (p-value > 0,05) and Pagel's lambda value was low ($\lambda < 0,2$). With these results we made a hypothesis that modern birds are now under the influence of stabilizing selection to save the remaining adenines. In this time we saw high numbers of A>G mutspec (~50%) in ancestors of diving birds. We think this is the time when birds accumulated the most amount of mutations, but this assumption needs further research.

We discovered that birds mtDNA is G-rich and its mutagenesis rate is much higher than in mammals. We found several life-history traits which increase mtDNA mutagenesis — getting ability to dive and migration type. But one factor can lower it — losing the ability to fly. We suppose that all mutagenesis effects erupted at the level of the ancestors and nowadays modern birds are under the influence of stabilizing selection. To continue our research we expanded our data by gathering mtDNA information from the MIDORI2 database for more than 7000 bird species [6]. In addition we also got A>G spectra for 789 birds by using the NeMu pipeline [7].

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