Project Title: Comparative Mitochondrial Genomics of the Dodo (Raphus cucullatus): Evolutionary Insights into ATP Synthase Genes

Project Summary: This project explores evolutionary changes in mitochondrial genes of the extinct dodo (Raphus cucullatus), focusing on ATP synthase genes (ATP6, ATP8), CYTB, and other key mitochondrial genes (COI, ND2, ND3, ND5), and compares them to a selection of both volant and flightless birds. The objective is to determine whether any molecular signatures of selection or divergence in energy metabolism are associated with the dodo's flightlessness. Our findings are discussed in the context of mitochondrial evolution, protein structural tolerance, and the potential transition to nuclear-encoded mitochondrial gene studies.

Objectives:

1. Extract and align mitochondrial protein-coding genes from 8 avian species.
2. Construct phylogenetic trees to evaluate evolutionary clustering.
3. Identify codon sites under positive (diversifying) selection using FEL, MEME, and other tests.
4. Model protein structure using AlphaFold and assess functional impacts of amino acid substitutions with SIFT and PROVEAN.
5. Prepare to transition to nuclear-encoded mitochondrial gene analysis.

Species Included:

- Raphus cucullatus (Dodo)
- Caloenas nicobarica (Nicobar pigeon)
- Columba livia (Rock pigeon)
- Streptopelia decaocto (Eurasian collared dove)
- Gallus gallus (Chicken)
- Apteryx owenii (Little spotted kiwi)
- Dromaius novaehollandiae (Emu)
- Struthio camelus (Ostrich)

Methods:

- Mitochondrial GenBank records were parsed using Biopython.
- ATP6, ATP8, CYTB, COI, ND2, ND3, ND5 genes were extracted where available using flexible gene annotation matching.
- Sequences were aligned in MEGA X (MUSCLE codon alignment).
- Phylogenetic trees were created using NJ + Bootstrap (500).
- Selection analyses were performed on DataMonkey (FEL, MEME, SLAC, FUBAR).
- PROVEAN and SIFT were used for predicting the effects of mutations.

- AlphaFold via Google Colab was used for structural modeling of dodo and Nicobar pigeon ATP8 proteins.
- PyMOL was used for structural visualization and residue labeling.
- COI was included as a high-confidence mitochondrial gene linked to oxidative phosphorylation, though no positive selection was found.

Protein Impact Predictions:

- **ATP8 Proline→Threonine** (codon 42): Predicted as *tolerated* by SIFT (score 1.00).
- **AlphaFold prediction** shows this site lies in a low-confidence region (plDDT < 70), likely a disordered terminus.
- PyMOL mapping confirms structural tolerance.

Conclusions (So Far):

- No strong evidence of functionally significant adaptation was found in mitochondrial genes of the dodo.
- Despite clustering differences and minor amino acid shifts, no impactful structural or conserved mutations were detected.
- Two genes (ATP8 and CYTB) had suggestive patterns of divergence, worth highlighting as weak signals.

Key Takeaway: While mitochondrial gene changes do not strongly support the initial hypothesis, their neutrality or relaxed selection is itself an informative result. Deeper genomic integration (nuclear-mitochondrial crosstalk) is now the logical next phase.