

## Bird proteins from long time ago

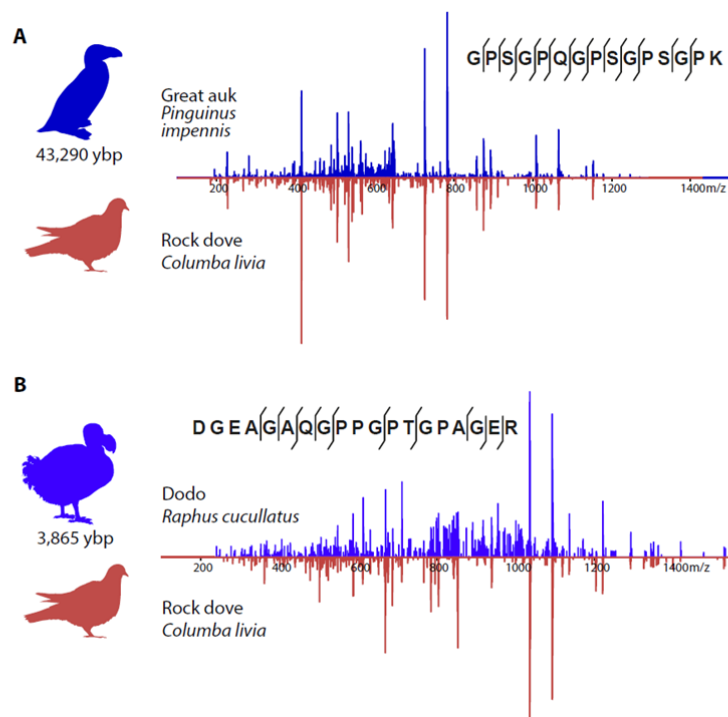
A recent field of biological research is termed 'palaeoproteomics', which aims at determining all proteins in very old biological samples. In this study, we compared bone proteins from extinct and present-day birds using palaeoproteomics and established the biological relationships between them.

The methodology used in palaeoproteomics allows investigators to study samples that are many millions of years old. These materials cannot be studied by looking at DNA, since palaeobiologists have shown that DNA is degraded after 1.5 - 2 million years. In palaeoproteomics, researchers analyse collagens – proteins involved in bone structure that are well protected in this environment from external influences – to determine species and relationships. The methods have been applied successfully on dinosaur bones by other groups.

We obtained the various bird bones from museums (Naturalis Biodiversity Center, Natural History Museum Rotterdam, the Netherlands), a supermarket and a pigeon fancier. Applying mass spectrometry, a key technique in this field, we determined all molecules in the samples. To extend the study, we included old chicken materials found in a cave in Indonesia and compared these to fresh chicken bones. We treated the bones for many days with various chemicals (hydrochloric acid and others) and determined the concentration of retrieved proteins. Then, after some processing steps, we injected these samples into the mass spectrometer and analysed the data using specific software, databases on the internet and in some cases complete sequence information from collaborating institutes.

We found substantial parts of the proteins in the analyses: up to 60% for some of them. Using this protein-focused approach, we were able to confirm earlier relationship studies based on DNA analyses. Also, we found certain changes ('deamidations') in the proteins as a result of influences from outside and that happen over time.

We concluded from these studies that we can determine bird relationships by investigating very old bird bones and that dodo is related to current-day pigeons and great auk to razorbill. The analyses show again that identification based on proteins can be a good alternative for DNA determinations.



## Original summary of the published paper:

We used proteomic profiling to taxonomically classify extinct, alongside extant bird species using mass spectrometry on ancient bone-derived collagen chains COL1A1 and COL1A2. Proteins of Holocene and Late Pleistocene-aged bones from dodo (*Raphus cucullatus*) and great auk (*Pinguinus impennis*), as well as bones from chicken (*Gallus gallus*), rock dove (*Columba livia*), zebra finch (*Taeniopygia guttata*) and peregrine falcon (*Falco peregrinus*), of various ages ranging from the present to 1455 years old were analysed. HCl and guanidine-HCl-based protein extractions from fresh bone materials yielded up to 60% coverage of collagens COL1A1 and COL1A2, and extractions from ancient materials yielded up to 46% coverage of collagens COL1A1 and COL1A2. Data were retrieved from multiple peptide sequences obtained from different specimens and multiple extractions. Upon alignment, and in line with the latest evolutionary insights, protein data obtained from great auk grouped with data from a recently sequenced razorbill (*Alca torda*) genome. Similarly, protein data obtained from bones of dodo and modern rock dove grouped in a single clade. Lastly, protein data obtained from chicken bones, both from ancient and fresh materials, grouped as a separate, basal clade. Our proteomic analyses enabled taxonomic classification of all ancient bones, thereby complementing phylogenetics based on DNA.

(The lay summary was written and peer-graded for a course on writing in the sciences; Stanford University.)