Recovering the DNA of an extinct species

The Ancient Aurochs

RYSZARD SŁOMSKI
Poznań University of Life Sciences
Institute of Human Genetics, Poznań
Polish Academy of Sciences
PAS Committee on Human Genetics and Molecular Pathology
slomski@up.poznan.pl
Prof. Ryszard Słomski is a pioneer of DNA diagnostic research in Poland, working on molecular diagnostics of genetic diseases, the characteristics of new human genes, biotechnology, and forensic medicine

Today's technology makes it possible to analyze genetic samples up to a million years old. Perhaps one day the aurochs will make a comeback to Poland's forests, thanks to Polish scientists

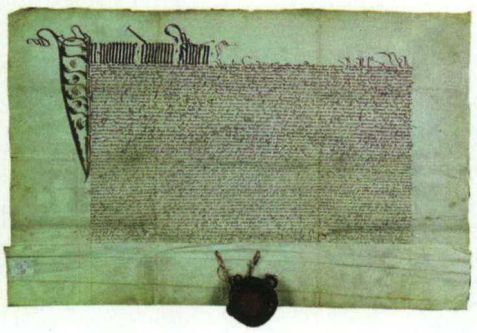
The aurochs - a now-extinct species akin to other large ruminants, such as the African buffalo, water buffalo, muskox, yak, bison, or wisent (*żubr*) - is thought to have been the ancestor of almost all European and African domestic cattle and also the cattle of continental Asia, including the zebu, the gaur, and the half-mythical kouprey cattle. The history of the aurochs is estimated to stretch back about 1.5-2 million years. It is widely assumed that the animals originally derived from the territory of India, from where they dispersed towards Asia Minor and subsequently further deep into Asia and to Europe. It is estimated that the aurochs reached Europe about 250,000 years ago.

The factors involved in the extinction of the aurochs presumably included human hunting and habitat pressure caused by the spread of farming. The species held out the longest in the very heart of the European continent - in Poland. Its period of decline came in the Middle Ages, and by the 13th century the aurochs survived in a few pockets of territory in Poland, Lithuania, Prussia, Moldova, and Transylvania. It was still attested in Lithuanian and Prussian lands in the 14th century but disappeared there immediately thereafter, leaving the last heard remaining in Poland's Masovia region, in the Jaktorów Primeval Forest. Even though King Sigismund I the Old took this last-surviving last aurochs herd under his special protection, providing yearlong care and feeding, the animal ultimately made its exit from history when the last cow died in 1627 near Jaktorów, having outlived the last bull by 7 years.

Historical accounts

A description of the species can be culled together and deduced from several sources. The first of these are pictures, reliefs, and cave paintings from ancient times, some of them dating to about 17,000 years back (or more). They are known from caves across almost all of Europe, from Northern Africa, Asia Minor (Anatolia), and Central Asia. The second source is a series of quite rare descriptions attested through history, including Sumerian, Egyptian, Greek, Roman (from the time of the conquests of Gaul and Germania), and Germanic (e.g. in sagas and songs, written in the runic alphabets) sources, plus also modern descriptions, mainly German and Swiss, which describe aurochs in the Polish and Baltic lands and also in Moldova. The most reliable accounts, however, are the Polish ones, especially from the 15th-17th centuries. The third source, quite limited although perhaps the most objective, consists of the physical remains of animals found in Europe, Africa, and Asia.

One of the famous historical monographs by Julius Caesar (100-44 B.C.), *Commentaries on the Gallic War*, included a description of the aurochs being hunted by Germanic peoples.



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Samantha Villagran, www.sxc.hu

Example of an aurochs skull found in Polish territory, from the collection of the National Museum of Natural Environment and Hunting in Uzarzewo. Aurochs remains are often found in Poland

The Swiss naturalist Conrad Gessner, in his great zoographic work covering all the quadrupeds, birds, fish, snakes, and scorpions known in his day, published letters by Anton Schneeberger and Johann Bonar providing substantial information about the aurochs. It is debatable, however, whether this information in fact comes from first-hand observations and experience, such as the claims that "this is a very agile animal, although not long-lived; they say that only a few of them have lived longer than 15 years" and that "they gather in September strongly excited and often wage battles against one other in which sometimes both sides fall dead." The modern-day researcher Cis Van Vuure (2005) casts doubt on this latter assertion, arguing that the estrous cycle more probably fell in August. However, his reasoning is based on an analogy to domestic cattle husbandry, which may not necessarily be convergent.

The physical remains of the aurochs that were once in the possession of many Polish museums were often considered unattractive, too "new" to be of interest, and were therefore kept stored away or handed over to schools. As a result, today it is actually hard to put together a full aurochs skeleton, which is a rarity.

However, the aurochs has left behind other, non-physical traces. In Poland and elsewhere there are many villages, towns, and places whose names are strongly connected to the aurochs. For instance, the name of the settlement of Turek in Poland has sparked certain controversies (the Polish word *tur* means "an aurochs," whereas the word *turek* can mean either "a small aurochs" or "a Turkish person"). Leon Lubomir Kruszyński wrote at the beginning of his 1892 *Monograph on Turek Town* that the name of Turek derived from the word *tur* (an aurochs), which he sees as synonymous with *żubr* (a wisent). Today we know that the name Turek indeed comes from the word *tur*, meaning aurochs. Some historians connect this place-name with a reference to Turcoviste found in the bull of Pope Innocent II, published in Pisa in 1136.

Description and research

The aurochs was definitely larger than today's cattle. It is thought to have been an enormous animal about 180-190 cm (bulls) or 160-175 cm (cows) tall at the withers - Julius Caesar even wrote of them as being "a little below the elephant in size." Bulls ranged in color from very dark auburn to black and had a lighter stripe along their back, while cows ranged from red to auburn. The color of the calves was lighter, irrespective of sex. In the case of cows the color changed only slightly, while the color of bulls visibly began to grow darker at about half a year of age.

Certain morphological features of various modern-day breeds of domestic cattle resem-

ble the respective features of the aurochs. This is particularly true among what are known as the primitive breeds, such as the Hungarian and Podolian steppe cattle, Scottish cattle, and breeds of Spanish cattle used in bullfights.

Scientific research on the aurochs has a history that stretches back to the late 18th and early 19th centuries. In 1827 Bojanus described an aurochs skeleton, coming to the conclusion that the animal was a separate species. He gave it a systematic name in Carl Linnaeus' classification system - *Bos primigenius* (Fig. 3).

The latest strand of research, which we are involved in, now strives towards reconstructing the DNA of the extinct animal.

Reading ancient DNA

The first research to work with such ancient DNA (aDNA) of extinct animals was performed by Higuchi in 1984, showing that genetic material could be extracted from a 150-year-old quagga (an extinct zebra-like animal) specimen and then sequenced. Success in this pioneering research was contingent upon obtaining relatively large quantities of good-quality DNA. The introduction of the polymerase chain reaction (PCR) into laboratory work made it possible to amplify DNA in vitro and thus popularized aDNA research, facilitating work with genetic material partially damaged by environmental factors. The term aDNA has been broadened to encompass all DNA obtained from dead organisms or their parts, including from museum collections and from tissues preserved for scientific purposes. All aDNAs are affected by degradation processes, leading to their fragmentation and often total destruction.

In general, aDNA researchers are interested in three aspects. The first - probably the most important - is learning about the genetic information which is included directly in a particular material and also in its surroundings. The second, directly related aspect involves comparing DNA information among specimens from the same population. This contributes to ascertaining individual variability and relationships which play a key role in the evolutionary process. Thirdly, the history of an extinct species is also recorded in its genes, so aDNA study can excellently complement anatomical research and contribute to our understanding of the causes for the species' extinction.

Today's technology makes it possible to analyze samples up to a million years old, although the most reliable results are obtained for samples not older than 100,000 years. The conditions in which the material was stored is another crucial factor. Our own experiences show that even a several-year-old sample may prove useless for DNA research if it is from a damp location.

The isolation of the aDNA itself is undoubtedly one of the most important stages. The success of further work crucially hinges upon this stage. Initially, material is prepared for study by removing external impurities, including also any genetic material from persons handling or studying the remains. Next deep boreholes are made, often with the assistance of dentists, in order to reach the best layers of material. In the case of aurochs remains this may be the cornual processes, which were covered by horny sheaths. To protect the aDNA so obtained and at the same time preserve it for future generations of researchers, it can be amplified in the laboratory by a method known as whole genome amplification (WGA). This procedure inserts the diverse DNA fragments obtained into plasmids that can be further amplified in bacteria and used to study the DNA structure. The next very essential stage of work involves sequencing the aDNA- learning about the order of DNA bases, or reading the genetic code. This stage of research is done using very technologically advanced equipment.

Focusing on mitochondria

In our work on aurochs aDNA, for comparative purposes we have also collected samples of domestic and wild cattle, isolating mitochondrial DNA (mtDNA) from aurochs (*Bos primigenius*), domestic cattle, Hungarian steppe cattle,

Ryszard Słomski together with Daniel Lipiński, preparing ancient bone specimens for DNA isolation

In many historical descriptions, the aurochs is typically confused with the wisent (*żubr*). Drawings of an aurochs and a wisent by Chodźko

Ukrainian steppe cattle, Heck cattle, banteng (*Bos javanicus*), zebu (*Bos indicus*), yak (*Bos grunniens*), bison (*Bison bison*) and wisent (*Bison bonasus*) specimens. Most archeological remains do not contain DNA traces that can be isolated by the methods commonly used to work with contemporary material. Ancient DNA is most often degraded, and so DNA molecules occurring in many copies per cell are easier to obtain for research. Each cell in diploid organisms like the aurochs contains just two copies of the nuclear DNA but hundreds or thousands of copies of the mtDNA, which can therefore be relatively easily isolated and subjected to sequencing even from archaeological remains.

A mitochondrial genome has many features that make it useful in molecular analyses. It is characterized by not large size (16,569 base pairs in humans), is inherited only in the maternal line, and evolves relatively quickly. The frequency of mtDNA mutation is 5-10 times higher than for nuclear DNA, and so it may yield information about the evolution of species that parted ways relatively recently. However, the mtDNA represents a separate genetic locus which probably does not reflect the history of the genome as a whole. This must be borne in mind, especially if research pertains to the genetics of a population or a range of closely related species.

One of the earliest achievements made by the present authors was to characterize the mitochondrial DNA (mtDNA) from a tooth of a man living 3000 years ago from the collections of the Museum of Warmia and Masuria in Olsztyn. We have since turned our attention to the aurochs.

Back to life?

Our work towards the possible restoration of the aurochs has already yielded many results, even within the short period of two years. One of the most important achievements has been to encourage public discussion about the aurochs, and in this sense the animal has already come back to life. Broader attention has moreover been drawn to the necessity of protecting free-living animals and to the great difficulties involved in studying extinct species. DNA research will enable us to identify the closest relative of the aurochs. This research of course still has a long way to go, but we can already illustrate some of the discovered mtDNA sequence similarities and differences with other species.

Polish scientists have a special right, or even duty to study the history of the aurochs using the most modern techniques. Attempts have been made to restore other species that once lived in Poland: the almost extinct population of wisent (*Bison bonasus*) and the "Konik" Pony or Polish Little Horse (*Equus caballus*) have been more or less successfully restored. Perhaps one day the aurochs will make a comeback as well. ■

Further reading:

The research described herein is being done by the team of: Ryszard Słomski, Alexander M. Dzieduszycki, Daniel Lipiński, Marlena Szalata, Joanna Zeyland, Łukasz Wolko, Karolina Wielgus, Mirosław S. Ryba.

Van Vuure, C.T. (2005). Retracing the Aurochs: History, Morphology and Ecology of an Extinct Wild Ox. Pensoft Publishers. Sofia-Moscow.