ANCIENT PIG DNA STUDIES RELEVANT IN EUROPE DOMESTICATION - REVIEW

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Abstract

Ancient DNA has been part of research since the early 1980s. With the improvement of molecular-genetic technologies, the areas of aDNA research have gradually expanded. A large increase in genomic analyses of historical pig finds began after 2000 and peaked in the number of published papers since 2015. Research most often focuses on the analysis of mitochondrial aDNA and the results are used to interpret domestication, phylogeny or migration. With the development of sequencing methods, whole genome analyses are naturally also appearing in studies, giving rise to comprehensive studies. This review provides a brief overview of the most thematically important articles in terms of European domestication. Due to the breadth of the topic, the review does not deal with research on domestication in Asia.

Key Words: aDNA; palaeogenomics; palaeogenetics; pig domestication

Ancient DNA

Molecular archaeology is a scientific discipline that involves the analysis of ancient biological material older than 75 years (GRAHAM, 2007). DNA derived from historical remains is therefore called ancient DNA (aDNA). Ancient DNA has many typical characteristics that make it difficult to analyse. These typical characteristics appear after the death of an organism as a result of decomposition and, over time, other endogenous and exogenous influences. These factors subsequently cause DNA damage. Mechanical damage is mainly caused by hydrolytic and oxidative processes, which lead to DNA fragmentation, the formation of abasic sites, and *postmortem* mutations (HOFREITER *et al*. 2001, WILLERSLEV and COOPER, 2005). The length of an aDNA fragments is typically between 50 – 200 bp (HOFREITER *et al*., 2015). In addition to frequent *postmortem* mutations, aDNA molecules also contain thymine dimers and crosslinks, which also complicate analysis (WILLERSLEV and COOPER, 2005). Another factor makes analysis difficult is the presence of inhibitors, which are isolated together with aDNA (SUTLOVIĆ *et al*., 2007). The aDNA samples are also highly susceptible to contamination with recent DNA (COOPER and POINAR, 2000). All these characteristics make it difficult to analyse biological samples from historical excavation.

The aDNA analyses generally target two distinct parts of the genome. Although nuclear DNA (nuDNA) carries all the genetic information about an individual inherited from both parents, it is unfortunately more susceptible to harmful processes. On the other hand, mitochondrial DNA (mtDNA) is inherited through the maternal line, exists in many copies in each cell, and, thanks to its circular structure, is more resistant to the passage of time. Therefore, mitochondrial aDNA widely used in aDNA studies. Recent improvements in extraction methods, as well as next-generation sequencing, enable the study of entire ancient genomes and provide valuable information about species and domestication (FRANTZ *et al*., 2020). Thanks to improvements in sequencing technologies ancient genomics has emerged (LAMBERT and MILLAR, 2006).

Ancient DNA research began in the 1980s, when DNA was successfully extracted from animals and humans for the first time. In the 1990s, however, what was originally a fantastic scientific discovery turned out to require special procedures for analysing aDNA. The research that caused a scientific stir in 1994 was an analysis of 80-million-year-old dinosaur DNA (WOODWARD *et al*., 1994), which turned out to be an analysis of recent contamination. This scientific error led to the definition of strict anti-contamination procedures in laboratories analysing aDNA (COOPER and POINAR, 2000). Currently, the oldest successfully analysed samples come from permafrost, such as a million-year-old mammoth (VAN DER VALK *et al*., 2021) or two-million-year-old remains from environmental DNA (eDNA) (KJÆR *et al*., 2022). The oldest human aDNA comes from the Sime de los Huesos cave in Spain and is 430 000 years old (MEYER *et al*., 2016). The first animal analysed using aDNA was not a domestic animal, but one of the horse's extinct relatives, the quagga (*Equus quagga quagga*) in 1984 (HIGUCHI *et al*.). The mitochondrial aDNA came from skin tissue from a museum specimen from the late 19th century. It was the first successfully extracted aDNA sample in history. This research can be considered the beginning of a new scientific discipline, molecular archaeogenetics.

Regardless of the difficulties, since the early 1980s, aDNA molecules have become an important source of information for a wide range of scientific disciplines, from archaeology, anthropology, anthropogenetics, to archaeozoology and archaeobotany. The possibility of researching ancient biomolecules, including DNA, has enabled the emergence and development of a whole new era of palaeogenetics and palaeogenomics.

Ancient pig DNA

Many research focuses on demographic, phylogenetic and evolution studies that aimed to recent pig DNA (e.g. FRANTZ *et al*., 2016, GROENEN *et al*., 2012), but ancient DNA studies can shed new light on extremely complex evolution. Table 1 lists selected, most relevant studies that mainly analysed aDNA from European historical pig remains.

GIUFFRA *et al*. (2000) concluded that the time since divergence of the ancestor of European and Chinese Meishan domestic pigs is 500 000 years BP. Furthermore, analyses of mitochondrial and nuclear genetic markers of recent domestic and wild pigs from Europe and Asia have demonstrated the existence of two independent centres of pig domestication.

Genomic samples provide evidence of two independent centres of pig domestication from two geographically and genetically distinct wild populations in western and eastern Eurasia (GIUFFRA *et al*., 2000, GROENEN *et al*., 2012). The existence of multiple centres of pig domestication in Eurasia has been confirmed by recent DNA analysis (LARSON *et al*., 2005).

Before the development of aDNA analysis techniques, zooarchaeological evidence of pig domestication showed that wild pigs were domesticated in Southwest Asia around 8 500 BC (ERVYNCK *et al*., 2001, CONOLLY *et al*., 2011, FRANTZ *et al*., 2016). Earlier archaeological findings thus indicated that the origin of the first domesticated pig was in Southwest Asia (LARSON *et al*., 2005). Genetic studies have confirmed that the oldest domesticated pigs in Western Eurasia originated from wild pigs from the Middle East in Anatolia approximately 10,000 years ago (OTTONI *et al*., 2013, FRANTZ *et al*., 2019).

Later, they spread to the Middle East and were brought to Europe by early farmers. In 2007, LARSON *et al*. analyzed 221 ancient pigs from Western Eurasia with ancestors from the Middle East and proved that domestic pigs were brought to Europe during the Neolithic period via two independent routes at least as early as the 4th millennium BC. At the same time, local wild boars were also domesticated. European domestic pigs replaced pigs from the Middle East. They proved that early Neolithic domestic pigs were introduced to Europe from Anatolia in the middle of the 6th millennium BC. At the beginning of the 4th millennium BC, they then reached the Paris Basin. The samples came from archaeological sites across Europe, including 12

samples from the Homolka site in the Czech Republic, which were dated to the Eneolithic phase 2700–2300 BC. Interesting findings were brought by KRAUSE-KYORA *et al*. (2013); hunter-gatherers in northern Germany acquired domestic pigs, and they were present there 500 years earlier than previously thought.

Domestic pigs arrived in northern Europe around 5 000 BC (CALIEBE *et al*., 2017). European wild boars were also domesticated during the same period, probably in response to the introduction of domestic pigs from the Middle East (LARSON *et al*., 2007). Mitochondrial haplotypes showed genetic turnover between 4 000 and 3 000 BC in northern Europe, which was the result of crossbreeding between local wild sows and domesticated boars from the Middle East (CALIEBE *et al*., 2017). This crossbreeding was uncontrolled and occurred as a result of free-range pig farming. Local wild populations were also better adapted to environmental factors and pathogens.

LARSON *et al*. (2019) again demonstrated that the first domestic pigs originated from wild pigs from the Middle East, which spread to Europe with the first farmers about 8,000 years ago, but subsequent gene flow with European wild pigs led to an almost complete genetic replacement of the ancestors of European domestic pigs within 3 000 years. The Middle Eastern ancestors thus disappeared almost completely as a result of gene flow from wild boars. LARSON *et al*. (2005) demonstrated through analysis of recent mitochondrial DNA from wild and domestic pigs that the origin of modern European domestic pigs is more likely to be European wild boar than Middle Eastern.

In addition to studies of domestication and phylogeography, pig aDNA samples are also used in research as evidence of historical events. One of the most interesting studies concerns a unique archaeological and historical site, the town of Hallstatt in Austria. HAMMER *et al*. (2018) provided unique analysis of ten historic pig bones and teeth from the Bronze Age originated from the samples of Hallstatt. Hallstatt is important historic site for its history connected with the salt mining. The results of analyses of mitochondrial DNA suggest that variability in mitochondrial markers is comparable with recent wild and domestic pigs. They concluded that the Bronze Age Hallstatt pigs originated from large herds or from different husbandries. The results did not prove the wild/domestic origin of the pig.

Historically much younger pig remains analysed RAMÍREZ *et al*. (2015). The pig bone remains from the sixteenth century were excavated from cistern at Montsoriu castle, Spain.

Due to comparison mitochondrial and nuclear genome data with the recent genome of Iberian pig, Spanish wild boar and a Guatemalan Creole pig proved, that the historical pig individual is closely related with the extant Iberian pigs and to European wild boar.

Conclusion

This brief paper provides a basic overview of the most important publications relevant in Europe that have analysed samples of porcine aDNA. The sources listed include both fundamental research papers and very detailed review publications.

Table 1. Selected key publications on ancient pig DNA relevant in Europe

Publication	Type	Samples	Topic	Content
Larson *et al*., 2005	Re-search	aDNA	phylogeography, domestication	multiple centers of domestication
Larson *et al*., 2007	Re-search	aDNA	domestication, Neolithic spread	pig mitochondrial haplotypes over 13 000 years
Larson *et al*., 2010	Re-search	aDNA, recent DNA	phylogeography, domestication	genetic turnover in East Asian pig
Ottoni *et al*., 2013	Re-search	aDNA	phylogeography, domestication	mitochondrial haplotypes, early domestic pigs, Near Eastern Neolithic zone, western Anatolia, Neolithic expansion
Krause-Kyora *et al*., 2013	Re-search	aDNA	domestication, Mezolithic	northern Germany hunter-gatherers acquired domestic pigs; domestics were present there 500 years earlier than previously expacted
Larson and Burger, 2013	Review		domestication	domestication of animals in general
Meiri *et al*., 2013	Re-search	aDNA, recent DNA	phylogenetics	pigs from Bronze Age in Israel had haplotypes of modern and ancient Near Eastern pigs; complete genetic turnover
Evin *et al*., 2015	Re-search	aDNA	domestication, Neolithic spread	admixture domestic pigs and wild boars after migration, mitochondrial turnover
Frantz *et al*., 2015	Re-search	recent DNA	domestication	gene flow and selection during domestication
Ramírez *et al*., 2015	Re-search	aDNA	breed relationship, Iberian pig	sixteenth century pig from Spain, relationship between Iberian, European wild boar and ancient pig
Tetens *et al*., 2016	Re-search	aDNA	domestication, Mezolithic	*MC1R* in wild boar Mezolithic two samples
Frantz *et al*., 2016	Review	fossils, not DNA	evolution	comprehensive overview of Suidae evolution
Caliebe *et al*., 2017	Re-search	aDNA	domestication change	haplotypes in northern Europe; 5000 - 4000 BC dometicates genetically from south Europe, 4000 - 3000 BC domesticates of northern origin, largely from local wild boars
MacHugh *et al*., 2017	Review	aDNA	domestication	genetic basis of animal domestication; dispersal of livestock during the Upper Paleolithic and Neolithoc periods
Scheu, 2017	Review	aDNA	domestication	comprehensive review of animal domestication
Hammer *et al*., 2018	Re-search	aDNA	case study, Hallstatt pigs, meat industry	mitochondrial haplotypes shared between historic and modern demestics and wild boar
Frantz *et al*., 2019	Re-search	aDNA, recent	domestication, Neolithic spread	domestics about 7000 y BP Near Eastern ancestry, later near-complete disappearance of Near East ancestry due to gene flow from wild boars
McHugo *et al*. 2019	Review	aDNA	domestication, palaeogenomics	using palaeogenomics data for animal agriculture
Price and Hongo, 2019	Review	aDNA archaeological samples	domestication	comprehensive review of domestication and pig management
Frantz *et al*., 2020	Review	aDNA	ancient genomics	comprehensive review of domestication

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