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► **To cite this version:**

Eva-María Geigl. Palaeogenetics and Palaeogenomics to Study the Domestication of Animals. A. Mark Pollard; Ruth Ann Armitage; Cheryl A. Makarewicz. Handbook of Archaeology, 2nd Edition, Wiley, pp.657-676, 2023, 978-1-119-59204-4. 10.1002/9781119592112.ch33 . hal-04162862

**HAL Id: hal-04162862**

**<https://hal.science/hal-04162862>**

Submitted on 16 Jul 2023

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# **Palaeogenetics and Palaeogenomics to Study the Domestication of Animals**

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The Latin word *domus*, house, is at the basis of the term “domestication”, chosen to describe the process during which animals and plants entered the human niche more or less intentionally promoted and/or directed by humans. Indeed, apart from dogs, domesticated over 15 000 years ago by hunter-gatherers, all other animals were domesticated once the nomadic lifestyle was abandoned and a semi-sedentary or sedentary lifestyle involving architectural structures, “houses”, was adopted. This was the beginning of one of the biggest adventures of humankind with profound repercussions on both the environment and humans that last up to the present day. The domesticated species considerably extended their distribution area, and often thrived while their wild ancestors went extinct, a process that is still ongoing (Geigl and Grange 2019). Thus, from an evolutionary point of view, the domestic form of most species is more successful than the ancestral form.

Since Charles Darwin (Darwin 1868), generations of archaeologists and biologists have been fascinated by domesticated animals and the study of the domestication process. These processes differed between domesticated species and their diversity was influenced by the physiology and behaviour of the wild ancestral species, the environment in which domestication took place and the societies that initiated it. While the domestication research of archaeologists focusses on the cultural side, i.e., the niches constructed by humans that allowed the recruitment of animals as commensals or source of primary (food) and secondary (e.g., milk, hide, wool, labour, etc.) products, archaeozoologists concentrate on the biological side, e.g., the osteometric analysis of animal remains in archaeological sites. The two approaches have yielded a plethora of results and a comprehensive view of the process of animal domestication in pre- and protohistory (e.g., Zeder 2012). Since the 1990s, this scholarly couple was joined by a newcomer: the field of palaeogenetics specializing in the analysis of DNA preserved in ancient remains (Pääbo 1989). Palaeogenetics, and even more palaeogenomics, the younger sibling of the field, enriched domestication research enormously, opening avenues

of research lines that will deepen and sometimes revolutionize our understanding of the processes of animal domestication (e.g., MacHugh *et al.* 2017, Frantz *et al.*, 2020).

## ANALYSIS OF DNA PRESERVED IN ARCHAEOLOGICAL SPECIMENS: WHAT ARCHAEOLOGISTS WANT TO KNOW

### **DNA extraction from archaeological specimens**

DNA preservation in biological material from archaeological sites is rather the exception than the rule. Depending on the climate in which the decay and diagenetic processes occurred, DNA can be preserved or not. As a rule of thumb, the hotter the climate is, the less DNA is preserved. Moreover, other factors play a decisive negative role, such as humidity, acidity of soils, soils through which water can percolate, fluctuations of the ground water level, or other less well-known factors (Pruvost *et al.* 2008). The taphonomic conditions that skeletons experience prior to burial are also important as they can determine whether DNA will be preserved. For example, the treatment of animal carcasses by humans can positively influence DNA preservation, in particular when body parts were cooked and quickly de-fleshed after death, as this can stop microbial growth (Pruvost *et al.* 2008). A second level of factors that contribute to DNA degradation are the post-excavation conditions. Indeed, DNA has been shown to be degraded after the exhumation of skeletal remains if washed and exposed to higher temperatures (Pruvost *et al.* 2007).

While most skeletal remains of vertebrates contain only minute amounts of endogenous DNA, the outermost layer of cortical bone has been reported to preserve DNA better (Alberti *et al.* 2018) as does the cementum part of carnivorous teeth (Adler *et al.* 2011). High amounts of DNA can be preserved in the petrosal part of the temporal bone (Gamba *et al.* 2014). In the comparison of DNA preservation in these various skeletal tissues, petrous bones are clearly the winners (Geigl and Grange 2018b). This discovery made petrous bones the privileged anatomical part for palaeogenomic analyses and led to their exponentially increased use. Unfortunately, petrous bones preserved in hot climates such as the Near East, which is of particular interest to domestication studies, often show poor DNA preservation. Moreover, since each individual has only two petrous bones, their availability is limited. In exceptional cases, DNA can also be very well preserved in other post-cranial skeletal remains, as it was the

case in a Denisovan and in a Neanderthal phalanx in the cave of Denisova from which high quality genome sequences could be obtained (Meyer *et al.* 2012, Prufer *et al.* 2017).

For a palaeogenetic analysis, only a small sample has to be taken from the archaeological bone or tooth material, roughly 50 to 100 mg. There are different sampling procedures and each laboratory has its own. One can sample by drilling into the bone, a procedure that produces bone powder and is used by many laboratories. Here it is important to know that the friction, which is produced during the drilling, produces heat that degrades DNA. Therefore, in our laboratory, we grind the bone sample that we have cut from the skeletal part using a Dremel saw in a freezer mill in liquid nitrogen, i.e., at  $-196^{\circ}\text{C}$ , to prevent DNA from being heated and degraded. The produced bone or tooth powder is finely ground and the increased surface allows efficient DNA extraction from the mineral part of the bone, a procedure that takes one to several days. The extracted DNA is then purified away from soil and bone substances that have been dissolved with the DNA. The purified extract is then either immediately amplified or transformed into DNA libraries that will also be amplified. In both cases, the DNA molecules have to be multiplied (amplified) to obtain enough material to be analysed by the current methods of molecular biology. The exact procedures followed can impact considerably the success and costs of ancient DNA analyses.

### **Methodological approaches: polymerase chain reaction versus next-generation sequencing**

The invention in 1985 of the polymerase chain reaction (PCR; Saiki *et al.* 1985) enabled molecular geneticists to analyse the DNA preserved in ancient animal remains, mostly skeletal and dental remains, but also skin, hair, hooves. This analysis is challenging since ancient DNA is highly fragmented and contains chemical modifications that change its coding information (Pääbo 1989). Moreover, only minute quantities of DNA are preserved in ancient remains. Thus, without multiplication of the ancient DNA molecules, an analysis with the presently available methods is impossible. Here, the PCR method provided a solution by multiplying billion-fold a specific target from a few ancient DNA molecules that had been extracted from an ancient specimen (Figure 1). This method led to a first blooming of the field of ancient DNA, before it became clear that the power of the method renders it very sensitive to contamination leading to the publication of numerous erroneous results, the most famous example being dinosaur DNA (Woodward *et al.* 1994). The sources of contamination are

diverse and can be found in both the archaeological excavation practices and the archaeological collection, but mainly in the palaeogenetic laboratory. Indeed, washing of excavated skeletal remains can lead to contamination with human, animal and plant DNA (Pruvost *et al.* 2007). Moreover, preparation of animal corpses for reference collections in proximity to archaeobiological collections can also lead to contamination of archaeobiological specimens (unpublished observation of the author). Most importantly, if not adequately controlled, laboratory procedures themselves lead inevitably to contamination with PCR-amplicons or cloned products. Finally, DNA contained in biochemical reagents (Leonard *et al.* 2007), such as the enzymes used to amplify ancient DNA, lead to the production of erroneous results unless biochemical decontamination methods are used (e.g., Champlot *et al.* 2010). This is of particular importance when human, bovine, porcine, rodent and bacterial DNA is analysed, as demonstrated through the analysis of more than 1,000 negative control reactions that produced phylogenetically meaningful DNA bovine and porcine sequences (see supplements to Champlot *et al.* 2010), as well as through compelling analyses showing contamination of reagents with rodent DNA (Erlwein *et al.* 2011, Kearney *et al.* 2012). This reagent-borne contamination renders many published results of ancient aurochs/cattle, wild boar/pig, mice and other rodents, as well as bacteria, such as *M. tuberculosis*, at least unreliable (e.g., Geigl 2008). A high-containment laboratory, although a necessary condition for ancient DNA research, is insufficient, in the absence of decontamination measures, to prevent the production of false-positive results due to contamination (Champlot *et al.* 2010). Through using methods to decontaminate reagents and PCR-products from previous experiments (Champlot *et al.* 2010), these contamination problems can be largely overcome but they rarely have been used in the field of palaeogenetics. This is regrettable since the high-throughput sequencing of amplicons produced in controlled, targeted multiplex PCRs of chosen genetic loci is a highly efficient, sensitive, powerful and financially affordable approach, that is particularly suited for archaeological material in which DNA is poorly preserved and not amenable to whole genome sequencing (Guimaraes *et al.* 2017). The contamination problem is less pronounced in studies based on next-generation sequencing (NGS) where PCR amplifications directly targeting the ancient DNA molecules are not performed (Figure 1). Instead, small DNA sequences, the so-called adapters, are ligated to the DNA molecules extracted from archaeobiological material and serve as the priming site for the amplification. This approach that has been used for the first time to produce a draft sequence of the Neanderthal genome (Green *et al.* 2010) is less prone to the production of artefacts due to contamination, although cross-contamination between samples can and does still occur. For very small amounts of preserved DNA,

contamination is a major issue. Furthermore, when sequence capture is used, the associated PCR amplification steps can produce high quantities of DNA molecules with limited sequence diversity that can also cause problems of carry-over contamination (Figure 1).

The reliability of the produced DNA sequence depends mainly on the degree of DNA preservation. Apart from the petrous bones mentioned above, which often contain high amounts of endogenous DNA, and a few, exceptional cases of post-cranial skeletal remains in which DNA is exceptionally well preserved, DNA is mostly poorly preserved in non-petrous bones. As a consequence, the genome sequences that can be retrieved from these bones are of low quality, i.e., the proportion of sequenced genomic regions is low. Moreover, due to the diagenetic lesions in ancient DNA that modify the genetic code, each nucleotide position of the DNA must be sequenced several times, ideally 30 to 50 times, to be reliably confirmed, a number that is reported in the publications as “fold coverage” or “X coverage”. This situation is rare, and so far, most ancient genomes published in the literature are of medium quality at best.

If DNA preservation is low, the coverage will be very low and therefore unreliable, and NGS is too expensive to warrant the cost of producing significant genomic sequences. Therefore, DNA enrichment procedures have been designed that capture targeted DNA sequences in solution (Gnirke *et al.* 2009, Haak *et al.* 2015, Massilani *et al.* 2016). While these methods are efficient, they limit the recovery of DNA sequences that deviate substantially from the DNA sequence of the organism used to design the probes with which DNA sequences in the ancient DNA extract are being captured. Moreover, they can potentially produce erroneous results due to cross-contamination with DNA barcodes between captured samples, which can, however, be minimized through the use of unique dual indexes (e.g., Rohland *et al.* 2015). Finally, certain DNA sequences, in particular those rich in the DNA nucleotides adenine and thymine, are difficult to capture and often need to be retrieved with targeted PCR.

Although achievable through whole genome sequencing, the enrichment of particular DNA sequences or their targeted amplification facilitates (and renders affordable) the analysis in archaeological specimens of mitogenomes or of specific genes and their variants involved in phenotypic traits, such as coat colour (e.g., Ludwig *et al.* 2009, Massilani *et al.* 2016, Guimaraes *et al.* 2017, Librado *et al.* 2017, Ottoni *et al.* 2017, Guimaraes *et al.* 2020).

## Phylogenetic and statistical analysis methods

Once DNA sequences from archaeological specimens are obtained, they are mapped on a modern reference sequence for their assembly. A special mapping process also reveals the damaged nucleotides in the DNA sequence (Ginolhac *et al.* 2011), a means of authentication for ancient DNA. De novo assembling ancient genomes, instead of aligning them to reference genomes, is notoriously difficult because of the extremely short size of DNA sequences. This is the reason why most ancient genomes are analysed through the alignment to reference genomes of closely related species.

The assembled and aligned genome sequence then have to be analysed using methods of phylogeny and population genetics. During the pre-genomic era, mostly the maternally transmitted mitochondrial DNA was analysed using PCR approaches. The obtained sequences were presented as median joining networks, a method that visualizes the proximity of DNA sequences (Bandelt *et al.* 1999) and allows phylogeographic inferences, such as origins of populations and their migrations, as well as bottlenecks. The reconstruction of phylogenetic trees is possible through phylogenetic methods, such as Maximum Likelihood methods (Guindon and Gascuel 2003, Stamatakis 2014). Moreover, Bayesian analyses allow the inference of the divergence times of tree branches (Drummond and Rambaut 2007). These analysis methods are also used to analyse partial or whole genome sequences. Indeed, often large numbers of single nucleotide polymorphisms (SNPs) are used to reconstruct population histories. Statistical methods are used to present the relationships between genomes and thus individuals. The principal component analysis (PCA) is such an instrument widely used in the ancient DNA field, in particular procrustes projection PCA (Wang *et al.* 2014). Here, the genome sequences of present-day individuals define the two dimensions of the PCA and the ancient genome sequences are projected, revealing their mutual genetic relationships. Patterson's D-statistic compares genetic closeness of four genomes: a test population's relative is compared to two other reference populations (Patterson *et al.* 2012). Numerous new statistical methods have been and are being published that increase resolution and power of the analysis of genomic data obtained from archaeological specimens (e.g., Bergström *et al.* 2020).

Using these model-based statistical methods one has to be aware of their limits and the biases they introduce as the underlying assumptions are, out of necessity, often over-simplifying the

analysed situations. The differential analysis of DNA sequences with various methods of population genetics can introduce artefacts, and even lead to contradictory results (e.g., Frantz *et al.* 2016, Botigué *et al.* 2017). Moreover, ancient DNA sequences being very short and damaged, enough sequence information to reliably cover a whole genome is rarely obtained. Thus, their analysis is biased and readers should be aware that there are pitfalls to these analyses, just as to any scientific approach. The combination of various approaches, i.e., palaeogenetics and -genomics, archaeology, archaeozoology and isotope analyses, helps to compensate for different biases of each one, some of which are partially overlapping. It is this interdisciplinarity that will allow us to make progress in the understanding of the domestication processes of the various animal species.

## PALAEOGENOMIC APPROACHES TO STUDY THE DOMESTICATION PROCESSES

How do palaeogeneticists proceed when they want to study the domestication of a species? It is likely that their interest in such a study is raised by the results of archaeological and archaeozoological studies. Based on these results, they probably construct one or several hypotheses that they then test through ancient DNA research. For this endeavour to work, they will establish collaborations with archaeologists and archaeozoologists with whom they can discuss the hypotheses, the rationale of their study and, at the end, interpret the results. Through these collaborations, the palaeogeneticists also receive the archaeological bones and teeth that they will analyse. Thus, ideally, a comprehensive and sound study needs a collaborative effort for the sake of the quality of the science produced and the impact it will have on the view of the analysed processes and on future studies.

Archaeologists collect material evidence that might indicate that animals were managed and then domesticated, such as enclosures, as well as dung and midden layers enriched in urine salts (Abell *et al.* 2019), to name just one particularly convincing example. Archaeozoologists propose ongoing or achieved domestication processes when they record a size difference between bones from wild and supposedly domesticated animals, a decrease in the degree of sexual dimorphism expressed in size and robustness of bones, as well as a change in the age and sex distribution of the culling pattern (e.g., Helmer *et al.* 2005, Abell *et al.* 2019).

What are the domestication signs palaeogeneticists will search for (Figure 2)? One reliable



indication of domesticated species that are not highly mobile is their range extensions (e.g., Zeder 2012). For example, wild sheep (*Ovis orientalis*) and wild goat (*Capra aegagrus*) were native to the Zagros mountains in present-day Iran and are considered to be the ancestors of the domesticated sheep and goats, but they most likely did not populate Europe in the Late Pleistocene and Holocene (e.g., Uerpmann 1987). The analysis of genetic markers in Neolithic bones from sheep and goats in Europe allows their unambiguous species assignment, often not achievable through osteometric analyses, and thus demonstrates their range expansion. This is, for example, possible through the analysis of mitochondrial DNA. Such an approach yields phylogeographic information of the periods prior to domestication, during domestication, and at the end of the spread of the domesticated forms. Accordingly, movements and spread of the domestic form along migration, trading and warfare paths suggest that the animals moved with the human migrants, merchants, soldiers and thus that the animal species integrated the human niche. This was the palaeogenetic approach that led to the discovery that cats conquered the ancient world as they accompanied Neolithic farmers as well as Greek, Roman and Viking sailors (Ottoni *et al.* 2017).

Apart from distribution shifts, geneticists search in the genomes of domesticated animals for changes compared to their wild ancestors. Since the human genome carries strong signatures of selection at variants underlying the phenotypic diversity of immune, metabolic and behavioural traits, it is likely that they are paralleled in the genomes of domestic animals. Therefore, geneticists try to find answers on questions such as how neoteny, i.e., juvenilization, a common characteristic of domesticated species, became fixed in the domesticated population. They compare the genomes of wild and domestic representatives of a species to find signatures that could be due to the novel anthropogenic environment the domesticates have entered, such as physiological changes due to captivity and changes in the nutritional composition of their food, increased exposure to infectious pathogens due to an increase in density in captive populations, social interaction with humans, etc. These questions can be analysed in the genomes of present-day animals, domesticated and wild, if the wild ancestor is extant. As an example, such an approach has shown that the genetic variant encoding for yellow skin in chickens originated from the grey jungle fowl (*Gallus sonneratii*) while data from other genetic loci identified the red jungle fowl (*Gallus gallus*) as ancestor of the domestic chicken (Liu *et al.* 2006, Eriksson *et al.* 2008). This demonstrated that present-day chickens draw their genomic ancestry from two ancestral species. If the ancestral form is extinct, one needs to adopt a palaeogenomic approach and analyse archaeological remains to answer questions about the

spatiotemporal origins of the domesticates, and of particular genomic signatures revealing environmental and anthropogenic selection pressures, as well as the ultimate question of what makes a domestic animal a domestic animal. The genomic changes that are analysed can concern individual genes, such as those involved or at least associated with certain coat colours. A striking example is the diversification of the coat colours that appeared in horse remains from Siberia and East Europe in the 5th millennium BP and increased in frequency during the Bronze Age (Ludwig *et al.* 2009). This was interpreted as the result of selective breeding, which is one of the hallmarks of domestication.

Populations adapt to new environments via selection on either new mutations or on standing, i.e., pre-existing, genetic variation. These two mechanisms have different evolutionary dynamics and distinct genetic outcomes. Compared with new mutations, adaptation from standing genetic variation is likely to lead to faster evolution, the fixation of more alleles of small effect and the more efficient spread of recessive alleles. The two mechanisms can be distinguished through the analysis of the genomic signature of selection. Indeed, genes or regulatory elements involved in certain metabolic functions, such as milk production and reproductivity in goats (Daly *et al.* 2018) or the ability of dogs to digest starch (Arendt *et al.* 2016, Bergström *et al.* 2020), are genomic target regions that are analysed for signs of positive selection. Such phenomena become visible when patterns of nucleotide divergence in a population are analysed and the diversity of single nucleotide polymorphisms (SNPs) in the genomic region encompassing the genetic variant under selection is reduced or eliminated compared to the surrounding genomic regions. If a new beneficial mutation is positively selected and reaches fixation rapidly, the linked variant can also become fixed, a process called a selective sweep.

Certain traits in animals are encoded by a single gene, i.e., a single locus in the genome. Other characters depend on several genomic loci, causing poly- or multigenic inheritance. The underlying alleles often affect phenotypes in a quantitative way and they are therefore called quantitative trait loci (QTL). Their analysis is more challenging than that of unique genes. A special array of physiological, morphological and behavioural traits are commonly observed in domestic animals and not shared with the wild ancestors, such as docility, smaller and often floppier ears, shorter muzzles, different shapes of tails, smaller teeth and an altered oestrous cycle. It has been suggested, based on experiments of foxes bred in farms (Belyaev 1979), that these characteristics are linked to neural crest cell deficits during embryonic development in a

phenomenon called “domestication syndrome” (Wilkins *et al.* 2014). Although these traits are not as universal as originally thought and each domesticated species harbours its particular suite of traits, their presence indicates that the selection pressure for these animals had changed, which can be due to adaptation to a particular anthropogenic environment and/or selective breeding (Lord *et al.* 2020). These changes could therefore also be the natural consequence of the complexity of the domestication process, which is different for each species. Since we are far from understanding well these complex and diversified processes, palaeogenomic studies are about to add substantial amounts of information that will help to decipher the biological side of these evolutionary processes of adaptation to the human niche.

In the following I will give a few examples of comprehensive palaeogenetic/palaeogenomic studies that have changed the way certain domestication pathways have been perceived, in particular the initial domestication processes, hybridization with wild ancestral species, as well as selection during later stages. I will follow a line combining both chronology and mechanisms referring to the three different pathways to animal domestication proposed by Melinda Zeder, i.e., the commensal pathway, the prey pathway and the directed pathway (Zeder 2012), that I will explain in the following based on examples. According to this model, the first animals entered the anthropogenic niche via the commensal pathway, in particular the wolf in the Upper or Late Palaeolithic. A second carnivore, the cat, probably followed later during the Neolithic through a similar scheme, the two ending up as human companions and playing important roles throughout history as they accomplished various tasks that shaped their relationships with humans, as well as their genomes. The wild boar is also thought to have been domesticated at the beginning of the Neolithic along the commensal pathway although it is a prey animal and not a companion, but its omnivorous feeding behaviour might have predestined it to approach human settlements as scavengers, just as wolves. In contrast, sheep and goats were the first herbivores to be domesticated at the beginning of the Neolithic through intensification of herd management. These species that occupied an important economic function in early and later sedentary societies were domesticated through captive breeding along the characteristics of the prey pathway. Finally, the later domestication of equids, first donkeys, then horses, the wild forms of which have been exploited for thousands of years, is considered to have been accomplished via the directed pathway based on previous experience with the domestication of other animal species.

## DOMESTICATION OF COMPANION ANIMALS

Carnivores, which had not been subsistence species, are thought to have been domesticated via the commensal pathway, as defined by M. Zeder (Zeder 2012). While commensalism might have been one of the driving forces of their domestication, the process finished with a nearly symbiotic relationship between them and their human owners.

### **Domestication of the dog**

Archaeological and archaeozoological research has shown that the wolf was the first animal humans domesticated, and the only large carnivore ever domesticated. Canid finds from *c.*12 000 year old Natufian sites on the territory of present-day Israel show signs of skeletal changes suggestive of both temperature- and domestication-induced adaptations (Davis and Valla 1978). Earlier finds in northern Europe dated to 31 700 years BP are discussed as potential early dogs, based on morphological criteria (Germonpré *et al.* 2009), although this conclusion has not found general acceptance in the scientific community (for a summary see Janssens *et al.* 2018). It is strengthened, however, by canid-like teeth finds from Siberia that are even older (Ovodov *et al.* 2011). These early “dogs” might have been wolves that scavenged on left-overs of meals in human camps. Stronger evidence for dog burials, known for almost 100 years and dated to around 14 700 years ago, is reported from Germany (Benecke 1987, Janssens *et al.* 2018). Genetic studies of present-day dogs and wolves have not resolved issues of the when and where of dog domestication. The analysis of wolf and basal dog lineages had narrowed the interval for the initial domestication of dogs down to a single event occurring 11-16 000 years ago, but other studies contradict this view (Vila *et al.* 1997, Savolainen *et al.* 2002, Freedman *et al.* 2014).

Presently, the domestication process of dogs is viewed as a slow and adaptive process in which wolves, natural competitors of Palaeolithic hunter-gatherers that shared the same environment and prey, approached human camps. Through this process, wolves would have become accustomed to humans. Since humans also killed wolves, it is conceivable that, in the case of killings of wolf mothers, the puppies would have been kept, ultimately resulting in their taming. This would have triggered an unintentional process of proto-domestication. Dogs have the cognitive abilities and temperament to excel at reading human cues. These traits, that would intuitively be attributed to dogs as the result of selection through domestication, seem to be

already present in wolves. Indeed, it has been shown that hand-raised wolf puppies spontaneously retrieved a ball for an unfamiliar person (Hansen Wheat and Temrin 2020). The authors conclude from this experiment that behavioural responses to human social-communicative cues are not unique to dogs but rather represent rare, standing variation in the expression of human-directed play behaviour in ancestral wolf populations that were a target for selective pressure in early stages of dog domestication (Hansen Wheat and Temrin 2020). Early socialization seems to be critical for these skills to develop (Udell and Wynne 2010).

Phenotypic traits are analysed in the genomes of present-day dogs (Lindblad-Toh *et al.* 2005, Parker and Ostrander 2005, Galibert and Andre 2008, Cadieu *et al.* 2009, Boyko 2011) and the dog became an excellent model for human genetic disease (Galibert and Andre 2006). The analysis of the copy number of the amylase gene *AMY2B* had been shown to be increased in present-day dogs as an adaptation to a starch-rich diet (Axelsson *et al.* 2013). This adaptation testifies for a profound physiological change that this carnivore underwent during the domestication process as it shared the cereal-based diet of an agricultural population. The analysis of the ancient dog genomes revealed that there are various copy numbers in Neolithic dogs suggesting that selection had started in the Neolithic. However, it was supposedly only in historical periods, starting in Sumerian Mesopotamia and Pharaonic Egypt and really expanding in Medieval and Renaissance times, that humans exerted more and more selective pressure to create breeds particularly adapted to specific purposes such as hunting, herding, rescuing, protecting (Boyko 2011).

The unresolved question of the origin of dog domestication has naturally attracted the attention of palaeogeneticists who started to search for an answer in the genomes of archaeological dog remains. Recently, several palaeogenomic studies of dog remains followed each other. Their genomic analyses took advantage of the genome of a 35 000-year-old wolf from northern Siberia (Skoglund *et al.* 2015) with which the ancient dog genomes could be compared in addition to present-day wolf and dog genomes. In 2016, an international team generated mitochondrial DNA and genome-wide SNP data from 59 European dogs dated to 14 000 to 3000 years ago as well as a genome sequence of good quality (covered at 28X, i.e., 28 DNA sequences per given genomic locus) of a 4800 year old Neolithic dog from Ireland (Frantz *et al.* 2016). The genome of this dog proved to be similar to present-day free-living dogs in terms of selection and breeding, and its capacity to digest starch was higher than in wolves but lower than in present-day dogs. The comparison of the genome-wide SNP data between ancient and

present-day dogs indicated that East Asian and western European dogs had separated ~14 000 to 6400 years ago, i.e., later than the appearance of dogs in the palaeontological and archaeological record. The Neolithic dog from Ireland clustered with the ancient western Eurasian dogs, but outside of the variation of present-day dogs and wolves. The authors then reconstructed the population history of East Asian and western Eurasian dogs using a population genetic method and software for inferring demographic history and population structure through time from genome sequences. This analysis revealed a reduction of the genetic diversity in western dogs, i.e., a genetic bottleneck that typically results when few individuals depart from a population. Taken together this would indicate that domestication took place in Asia more than 14 000 years ago, and that a small subpopulation of these dogs migrated west, probably accompanying migrating human groups (Frantz *et al.* 2016). Since the above mentioned at least 14 700-year-old archaeological canid remains in Germany suggest that dogs had already been domesticated in Europe when the Asian lineages arrived, the scenario proposed was that a replacement, albeit incomplete, of the old autochthonous European by the incoming Asian dogs took place, suggestive of a dual origin for dogs and thus two independent domestication events (Frantz *et al.* 2016).

A study of the genomes of an Early and Late Neolithic dog from the German site of Herxheim was published a year later, including the leading authors of the previously described study, yet that contradicted the previous one (Botigué *et al.* 2017). Indeed, this later study concludes with a much deeper divergence estimate between eastern and western dogs amounting to 24 000 – 17 000 years ago, with genetic continuity in western Europe and with shared ancestry between the Neolithic and present-day dogs. These data indicate a single rather than a dual origin of dogs. The difference between the two studies was attributed to a technical artefact in the population genetics analysis in the 2016 study. These Neolithic dogs from Germany had not yet developed the adaptation to a starch-rich diet.

The latest large-scale palaeogenomic study of dogs is in continuity with the Botigué *et al.* study of 2017. The authors sequenced a total of 27 ancient Holocene dog genomes from Europe, Siberia and the Near East (Bergström *et al.* 2020). This analysis showed that already in the Upper Pleistocene, dog lineages were diversified into five separate clades, the European, Asiatic, Near-Eastern/African, and the Arctic clade that interbred over large geographic distances. The authors found also genomic traces of interbreeding between wolves and dogs after their genomic divergence, but mainly from dogs to wolves. This unidirectionality could

reflect strong, possibly human-driven selection against hybrids or the effect of different population sizes, but could also be due to feralization of dogs. The ancient dog genomes distribute across a genetic cline in the population genetics analysis (outgroup  $f_3$  statistics) between East Eurasian and ancient Near Eastern dogs, indicating substantial admixture between these populations. The genomes of the earliest Neolithic European dogs reached the best fit in the modelling approaches with a dual ancestry, i.e., as mixtures of a Scandinavian dog associated with a Mesolithic context and Levantine dogs, in agreement with the genetic cline that was obtained in the population genetics analysis described above. Here again, no indication for multiple dog origins was found, but rather a single domestication event around the Last Glacial Maximum, possibly from a wolf lineage that is extinct at present and not yet characterized in ancient remains. The analysis of the gene encoding the starch-digesting enzyme amylase showed that some Neolithic dogs had as many copies as present-day dogs while others did not. This suggests that selection for increased copy numbers had not occurred at early stages of domestication and of cereal-based human life-style, but only began several thousand years later.

In Bergström *et al.*'s (2020) study, the genomes of contemporaneous humans and dogs were analysed in parallel. Results indicated that both dog and human populations in northern Eurasia prior to the Holocene shared circumpolar ancestry. It also revealed that Neolithic Near Eastern farmers migrated into Europe accompanied by their Near Eastern dogs, and that both humans and dogs admixed with the autochthonous Mesolithic farmers and their dogs, respectively. This became evident through the close genetic distance between a Swedish 10 900-year-old dog associated with a Mesolithic context and a dog from a 4800-year-old hunter-gatherer Pitted Ware Culture site in Sweden. A contemporaneous dog from a Swedish Neolithic agricultural context, however, shared closer ancestry to the Levantine dogs. Similar genetic relationships were found for humans from the same sites. In general, the Neolithic dogs in southern Europe proved to be genetically closer to Levantine dogs than dogs from northern Europe. During the Bronze Age, however, the dog genomes do not tell the same history as the human genomes. Most of the dogs from Middle Europe keep their close genetic distance to the Neolithic dogs, with a few exceptions, such as a dog associated with the Corded Ware culture, that share ancestry with a steppe dog. This suggests that the nomads from the steppes did not arrive in middle and western Europe with their dogs, maybe because they were on horseback? This is at present a pure speculation.

The Neolithic Levantine population did not contribute ancestry to present-day dogs in the area. Instead, there seem to have been a complete replacement of dog ancestry in the Levant around 2300 years ago, followed by gene flow from European dogs. Replacement of dog populations took also place in Europe after the Bronze age. Indeed, a dog from a Neolithic megalithic context in Sweden dated to around 5000 years ago and with a genomic composition half-way between the Mesolithic dog from Sweden and the dogs from the Neolithic Levant shared 90 – 100% ancestry with present-day dogs in a modelling analysis. This suggests that a population of this double genomic composition from the North and the South replaced previous populations and erased the continent-wide genetic cline. A few more recent dog genomes frame this event to a date between the Bronze and the Middle Ages. This European ancestry has spread around the planet and constitutes the major component in present-day dogs all over the world.

This comprehensive and fascinating study sheds light on the long relationship between humans and dogs that was closely interwoven over long time periods and large geographic distances, although exceptions exist. Nevertheless, the analysis still did not give an answer to the question of the initial domestication of the dog and further studies on additional archaeological specimens are needed to resolve this enigma.

### **Domestication of the cat**

Unlike present-day dogs, there is little phenotypic variation among cats of the genus *Felis silvestris*. The differences are so subtle that it is difficult to distinguish a stray cat not subjected to a breeding program from a wild cat. This is even more true for the determination of archaeological remains, which are scarce since cats were not a major subsistence species. Genetic studies of present-day cats produced an important contribution to the questions surrounding the domestication of the cat, as they showed that all domestic cats descended from the North African/Southwest Asian wildcat *F.s.lybica* and that admixture between wild European and domestic cats is a frequent event (Driscoll *et al.* 2007). It is this admixture, the level of which is unknown, that renders impossible a genomic investigation of the domestication process based solely on present-day or even historical specimens. The first step to elucidate this process was to investigate whether cat populations were structured prior to domestication. Based on archaeological research that had suggested an intensification of the human-cat relationship during the Neolithic in the Near East and later in Egypt (Vigne *et al.*



2004, Van Neer *et al.* 2014), it was assumed that the solitary, territorial wildcats overcame their natural fear of humans and approached settlements infested with rodent pests due to their stocks of cereals. Cats and humans became acquainted to each other, the beginning of a symbiotic relationship from which both partners benefitted: cats had access to an unlimited food supply, and humans got rid of venomous animals such as snakes and scorpions, as well as pests that spoiled their food stocks and destroyed whatever organic material was used in houses and ships (reviewed in Geigl and Grange 2018a). If this hypothesis were true, one would expect to see a shift in the population structure in Europe with the appearance in Neolithic sites in the Balkans of cats from the Near East, the initial Neolithic domestication centre.

Based on this rationale, we undertook a large-scale palaeogenetic study of cat remains in Europe, Southwest Asia and North Africa covering the last 9000 years to determine the phylogeographic pattern of mitochondrial DNA prior to the Neolithic migrations and its modification afterwards (Ottoni *et al.* 2017). The poor DNA preservation in most of the analysed skeletal material led us to choose the targeted and sensitive palaeogenetic approach that we had developed previously (Guimaraes *et al.* 2017). Our findings supported our initial hypothesis and showed that a mitochondrial lineage of *F.s.lybica* that we had identified in cat remains in Early Neolithic contexts in Anatolia was also present in Neolithic contexts in the Balkans, while cat remains from Mesolithic contexts in Europe carried the mitochondrial haplotype of the European wildcat *F.s.silvestris* (Figure 3). In Early Neolithic Southeast Europe, we identified another *F.s.lybica* lineage that was absent in the Near East and that diverged phylogenetically from the Anatolian lineage of *F.s.lybica* around 20 000 years ago during the Last Glacial Maximum when the Bosphorus was a land bridge. It is therefore reasonable to postulate that this lineage spread independently from humans prior to the Neolithic. In Egyptian cat remains from the 1st millennium BCE, we identified another mitochondrial lineage and could show that it spread within a few hundreds of years all around the eastern Mediterranean and up to the Baltic Sea, where we identified it in remains from a Viking port (Figure 3). The cat behind this mitochondrial lineage must have had particularly interesting features for humans since it replaced a large part of the local cats in Roman settlements in Anatolia. It was only after the Middle Ages that the proportion of this cat lineage among the domesticated cats dropped to its present-day level, which is roughly equal to the ancient Anatolian lineage. In a Roman harbour at the Red Sea shore of Egypt, some of the cat remains carried the mitochondrial lineage from another *Felis* subspecies: the wildcat from Central and South Asia, *F.s.ornata*. Historical documents describe an intense trading

connection of this port with India making a transfer of Indian cats or hybrids between *F.s.lybica* and *F.s.ornata* on sailing ships likely (Figure 3). These findings hint at sailing ships as major spreading vectors for cats, which again emphasizes the symbiosis between cats and humans, humans being ideal food providers for cats and cats ideal pest controllers for humans. They also hint at hybridization events taking place when ship cats met local wildcats. The conclusion of these results was that the world-wide spread of the cat from North African and Southwest Asian was the result of cats being the best companions of the sailors. Indeed, up to 1975 the British Navy prohibited ships to cruise the oceans without cats on board. The example of the dog and the cat shows that reconstruction of the spread of companion animals or commensal animals not only tells the history of the animals, but also parts of the history of humans!

The combined isotopic and palaeogenetic analysis of cat remains in Poland dated to the Neolithic period but found outside of Neolithic contexts has added another piece to the history of the spread and domestication of cats (Krajcarz *et al.* 2020). Through the analysis of stable isotopes in archaeological remains, it is possible to determine the organism's food sources. Nitrogen isotopes indicate the trophic level position of organism. Organisms higher in the trophic pyramid have accumulated higher levels of  $^{15}\text{N}$  (and higher  $\delta^{15}\text{N}$  values) relative to their prey and others before them in the food web. The mitogenomes of these cat remains were analysed using a capture approach. The obtained genetic data were interpreted based on the results of the preceding palaeogenetic study (Ottoni *et al.* 2017), thus allowing the assignment of the remains to European wildcats or Near-Eastern cats and to the temporal frame of their distribution. In contrast to pre-Neolithic and Early Neolithic European wildcats, the isotope signature in Late Neolithic and Roman cat remains indicated that they fed on animals with relatively high  $\delta^{15}\text{N}$  values. This was interpreted as an indication that these prey animals consumed cereal grains that have been manured, thus yielding  $\delta^{15}\text{N}$  values higher than those of carnivores feeding on wild animals. The contemporary human and dog remains of this study showed the highest  $\delta^{15}\text{N}$  values in agreement with the remains belonging to Neolithic individuals that relied on a carbohydrate-rich diet harvested from manured fields. The authors deduced from these results that the Late Neolithic cats thrived on synanthropic herbivores and omnivores foraging in agricultural areas as well as on wild forest herbivores and omnivores. Thus, these Late Neolithic cats in Poland were opportunistic synanthropes exploiting both the natural and the anthropogenic environments while the contemporary European wildcats occupied much wider isotopic niches. The latter experienced a shift of their isotopic signature owing to a shift in their prey species from wild forest herbivores and omnivores to more wild

omnivorous migratory birds and synanthropic rodents. This shift in the prey spectrum might reflect either the deforestation of the environment due to agriculture, and/or adaptation to a shift in the abundance of prey species, and/or the loss of prey due to anthropogenic pressure or even a change of the food sources of their prey. In conclusion, it can be hypothesized that while Early Neolithic cats still lived like wildcats, Late Neolithic cats adopted a lifestyle akin to the one of present-day feral domestic cats that do not depend on humans but remain in their neighbourhood and exploit synanthropic prey. This also indicates that incoming Neolithic Near-Eastern cats were not necessarily in competition with the autochthonous wildcats, but shared parts of their biotopes. It remains to be seen whether this can be generalized.

The analysis of a genetic variant for the coat pattern in cats showed that a pattern, the so-called tabby blotched, that is very frequent in present-day house cats but not in wild cats, appeared late in history, only after the Middle Ages (Ottoni *et al.* 2017). This is in agreement with the results of the analysis of the genomes of present-day cats where only few changes between the wild and the domestic cat have been identified (Montague *et al.* 2014). These changes concern mainly genetic loci involved in behavioural traits such as those concerning docility and sociability. This scarcity of differences between the genomes of wild and domestic cats, however, can also be the result of continuous hybridization between the two forms with the consequence that unadmixed wildcat genomes are not available. Thus, it would be necessary to sequence the genomes of archaeological cat remains from periods prior to higher-level admixture. This is the task pursued by the authors of this first large-scale palaeogenetic study that provides the framework for future palaeogenomic studies.

## DOMESTICATION OF THE FIRST LIVESTOCK SPECIES

The third species domesticated along the commensal pathway that I would like to discuss is the pig. Its position establishes the transition to the economic species domesticated as subsistence species.

### **Domestication of the pig**

Wild boar and pig (*Sus scrofa*) are omnivorous animals. Wild boars were hunted in Eurasia throughout pre- and protohistory. Archaeological and archaeozoological research suggests that

their domestication began around 10 500 years ago although there remains some uncertainty due to the difficulties of classification based on osteometric data, in particular during the early phase of the domestication process. Geometric morphometrics has eased this discrimination between wild and domesticated pigs and shown that the pigs introduced from the Near East into Europe were considerably smaller than European wild boars. Based on tooth size differences, all *S. scrofa* remains in Europe predating the Neolithic have been assigned to wild boar (Rowley-Conwy *et al.* 2012). The widespread distribution of the species, however, raises the possibility of more than one centre of initial domestication. Palaeogenetic studies have therefore tackled the domestication and spread of *S. scrofa*, starting with the analysis of the mitochondrial DNA. This research has shown that Neolithic pigs carrying the Near-Eastern mitochondrial haplotype reached northern France by *c.*6000 years ago (Larson *et al.* 2007, Ottoni *et al.* 2013). Around 100 years later, these Near-Eastern lineages had been replaced by the lineages of the European wild boars, hinting at introgression of the local wild boar populations into the domestic pig populations. If this process involved wild, captured sows and domestic boars, the offspring would carry the European mitochondrial haplotype. For a replacement of the mitochondrial lineage to take place efficiently, as apparently had been the case, it is likely that these hybrid offspring had phenotypic traits that made them more attractive to Neolithic farmers so that this practice became widespread and regular. Since mitochondrial DNA is only transmitted as a single genetic marker from mothers to their descendants, these data do not have the resolution to discriminate introgression from domestication. Therefore, genome-wide data of 54 archaeological specimens covering *c.*9000 years and two well covered genomic sequences were generated (Frantz *et al.* 2019). Their phylogenetic analysis yielded a result akin to the one obtained from the mitochondrial DNA analysis. They showed that prior to the Neolithic, wild boar populations in Europe and the Near East were structured. Present-day wild boars in Greece were shown to harbour up to 38% ancestry from the Near East, while Italian ones harbour only up to 10% (Frantz *et al.* 2019). This is most likely the result of migration and hybridization of Anatolian wild boar populations across the eastern Mediterranean forming an introgression cline. It could also be the result of the Early Neolithic migration of southwest Asian farmers along the coasts of the Mediterranean islands and peninsula, accompanied by their pigs with Anatolian ancestry. When these farmers settled hybridization of their pigs with local wild boars can be imagined as inevitable. As a consequence, a gradient of increasing European wild boar ancestry would have been formed from the East to the West. The latter process has been identified in Germany (Krause-Kyora *et al.* 2013). Present-day European domestic pigs were shown to have very low levels of Near-

Eastern ancestry, amounting to only ~4% and concentrated in only a few breeds that have not experienced admixture with Chinese breeds (Frantz *et al.* 2019). This study further revealed that Neolithic domestic pigs in Europe had strongly varying levels of Near-Eastern genomic ancestry, between 9 and 54%, indicating that incorporation of European wild boars into the Near-Eastern domestic pigs occurred rapidly upon migration into Europe. Taken together, the results of this study unveil a dynamic pattern of Near-Eastern genomic ancestry in European pigs gradually decreasing over time. In contrast, but not surprisingly, Bronze Age domestic pigs in Transcaucasia derive from Near-Eastern wild boars and did not experience admixture with European wild boars.

The screening in genomes of present-day pigs for signature of selection revealed that genomic regions under selective sweep had a closer genetic affinity to European than to Near-Eastern wild boars. This suggests that most, but not all, human-driven selection did not target genomic regions of Near-Eastern origin. A specific mutation in the Melanocortin A Receptor gene (MC1R), which is associated with black coat colour in western Eurasian domestic pigs, was not found in ancient and present-day wild-boars from the Near East and Europe, but in the majority of ancient domestic pigs that had at least one copy. The mutation was present in both Early Neolithic pigs from Anatolia and from Southeast Europe and thus most likely arose first in the Near East. Furthermore, it is likely that this mutation arose only once and was then maintained in domestic pigs despite the gene flow from wild boars that was detected.

Taken together, these results indicate that the wild boar was domesticated only once, in the Near East, followed by a long and complex history of management of pig herds that included (most likely intentional) interbreeding among different herds, as well as hybridization with the European wild boar. Present-day herding practices in the Mediterranean are often relaxed and include transhumance so that free ranging pigs naturally interbreed with wild boars. It is likely that similar practices were used in Neolithic times, leading to a profound genomic turnover in the domestic population that erased large portions of the original Near-Eastern genomic ancestry of pigs.

### **Domestication of food animals – goat and cattle**

With cattle and pigs, sheep and goat make up the quartet of food species that were the first to be domesticated by Early Neolithic societies about 12 000 years ago. The modalities of their

domestication processes have been described as the prey pathway (Zeder 2012). Their economic importance was long-lasting and never challenged by subsequently domesticated species. Sheep and goats shaped profoundly pastoralist societies up to the present. This is one of the reasons why the study of their remains is important when it comes to unravelling the transformations over time of lifestyles and societies. The other reason is that, unlike cattle and pigs, their wild ancestors were restricted to Southwest Asia and therefore could not have been domesticated in Europe. This eases the archaeozoological determination of their remains outside of the domestication centre and makes them very useful indicators of farming societies.

The wild progenitor of domestic goats, the bezoar ibex *Capra aegagrus aegagrus*, and of domestic sheep, the Asiatic mouflon (*Ovis orientalis*), were among the preferred prey species of hunter-gatherers in the Near and Middle East. These species are still living in the territory of present-day Iran allowing for genetic studies that compare their wild and their domestic forms (Alberto *et al.* 2018). These studies have shown the evolutionary path goats and sheep took since their divergence, and have identified their different deleterious genetic load and their similar population dynamics since domestication around 10 500 years ago in the Middle East. Moreover, numerous genomic regions have been shown to be under selection in the domestic forms compared to the wild ones, many of them in both sheep and goats but with different selection patterns. The regions under selection encompass genes related to pigmentation, immunity, productivity, hair quality, fertility and the nervous system.

Archaeological evidence from Southwest Asia suggests that sheep and goat had their own separate origins and dispersals, with multiple locations of domestication (Conolly *et al.* 2011). The initial phases of the transition from hunting to herding in Southeast Anatolia and the Zagros mountains in present-day Iran may go back 12 000-13 000 years (Zeder 2012). Domestic sheep and goat did not reach western Anatolia until the early 7th millennium cal. BCE (Çakırlar 2012) and from there the European continent. The spread within Europe followed the Neolithic migration routes, but there is no fine-scale comprehensive picture of the evolution of these species after their introduction in Europe.

Palaeogenetic work on early Neolithic caprines is sparse, but there are two major studies of mitochondrial DNA in archaeological goats (Naderi *et al.* 2008, Daly *et al.* 2018). Most Neolithic samples harboured mitogenomes that cluster with modern haplogroups A, B, C, D and G, while three pre-Neolithic samples constitute separate, more divergent clades F and T

together with the West Caucasian tur (*Capra caucasica*) and some Sicilian goats as well as the bezoar, respectively (Daly *et al.* 2018). In Early Neolithic domestic goat populations, these mitochondrial DNA lineages are clustered geographically: lineages A and C in the West (Anatolia and the Balkans), lineages B, D, G in the East (Transcaucasia and Northwest Iran) and lineage F in the South (Levant). They constitute 81% of the mitochondrial diversity. Modelling the divergence time of these clusters suggests a pre-Neolithic branching of the Levantine population. These results suggest multiple origins of Neolithic goat herds. This tripartitioning of the mitochondrial diversity disappears in post-Neolithic samples. Instead, haplogroup A becomes widespread and ubiquitous until now.

Genome-wide data from these specimens mirror the three mitochondrial clusters and support the conclusion of the mitogenome analysis that there was not a single common origin of domestic goats (Daly *et al.* 2018). Phylogenetic reconstructions based on these data suggest that the western and eastern branches separated in the Upper Pleistocene, more than 50 000 years ago. The eastern branch yielded the eastern Neolithic population while the genetic ancestry in the western and Levant Neolithic goats shows yet another component related to the Anatolian wild population. These different genomic components can be explained by substantial local recruitment into early domestic herds from various regional wild populations. These results indicate a protracted, multicentric domestication process spread out over a large geographic area. Such a process is in agreement with archaeozoological evidence for disparate management strategies in the different areas during the Early Neolithic (Horwitz *et al.* 1999, Arbuckle and Atici 2013). Moreover, palaeogenomic data from Early Neolithic humans also show that the transition from a hunter-gatherer to an agricultural lifestyle occurred regionally in the Levant, the territory of present-day Iran and Anatolia (Lazaridis *et al.* 2016). This implies that the various human communities acted somehow independently and exchanged their know-how and materials. The comparison between present-day goat genomes and Neolithic ones revealed western Anatolian ancestry in European goats, south-eastern Anatolian/Zagros ancestry in Far Eastern goats and predominantly Levantine ancestry in African goats, but enriched by ancestry from the other sources which might have introgressed during later periods.

Daly and collaborators also compared the genomes of both the eastern and western Neolithic goat populations with the genomes of present-day goats to search for selective sweeps testifying for adaptation to the anthropogenic niche resulting from different habitats, a different diet, different pathogens, but also human-mediated selection. The selection signature found in

goats from both Neolithic populations concerns the pigmentation loci *KIT* and *KITLG*, genes coding for a receptor tyrosine kinase and its ligand, respectively, that are still under selection in present-day goats. These genes are involved in the piebald phenotype and the authors speculate that they were selected to distinguish individuals and flag their belonging to specific owners, which might have been important in shared village herds. The signatures of the corresponding selective sweeps, however, are different in the western and eastern clades, and so are the resulting locus genotypes. In addition, these different genotypes contribute differently to present-day western and eastern populations. Several other genes have been identified as being under selection in the Neolithic goats analysed, such as those affecting stature, the calving interval, reproductive frequency, and mammary gland development linked to milk production. Finally and surprisingly, a gene was found to be strongly selected that is a homologue to a human gene involved in the detoxification metabolism, mainly of xenobiotics. Enniatin B is such a xenobiotic produced by fungi that grow on cereals and grains as substrate. This signal was interpreted as being the result of the recycling of agricultural byproducts as animal fodder, hypothesized as being one of the motivations for the origins of husbandry (Vigne *et al.* 2017). In such living conditions, fungal toxins might have been a general challenge for early domesticates and their farmers.

The fourth species of the group of Artiodactyla that have been domesticated during the Early Neolithic (PPNB) in the Fertile Crescent is the aurochs *Bos primigenius*. Archaeozoological evidence hints to southeast Anatolia as the area where, for the first time, sexual dimorphism, which is very pronounced in this species, was found to be reduced. This reduction is interpreted as the consequence of the management of this species (Helmer *et al.* 2005). In addition, a general size reduction and changes of the kill-off pattern and of the stable isotope signatures have been identified (Hongo *et al.* 2009). When genomes of supposedly wild and domesticated cattle remains from Anatolia and present-day Iran were analysed, they clustered together in a principal component analysis (PCA) based on present-day genomes onto which the ancient genomes are projected (Verdugo *et al.* 2019). Their position in the PCA identifies these genomes as the ancestral population of domesticated cattle. In this PCA, genomes of Neolithic domestic cattle from the Balkans are shifted toward genomes of present-day European cattle and the genome of the only European aurochs published so far, a 6738-year-old aurochs from southern Britain (Park *et al.* 2015, Verdugo *et al.* 2019). This shift can be explained through hybridization of Near Eastern cattle with European aurochs. The relative proximity of the cattle genomes from the Balkans to the Anatolian cluster demonstrates that it was this population that



migrated into Europe during the Neolithic. In contrast, after *c.*2000 BCE, the Levantine cattle experienced introgression from the Indian zebu, which erased the ancestral genomic signature. Contemporary cattle-zebu hybrids were also identified in Bronze Age and Iron Age Iran and Central Asia, indicating systematic introgression of Indian zebus that resist much better to aridity than European and Near-Eastern cattle. Thus, this finding might be the result of human-mediated adaptation of the cattle herds to a dryer, hotter climate, maybe linked to the 4.2 ka climatic event. This unexpected result adds an important piece of evidence to the research on the domestication process of the aurochs that is still elusive and requires substantial further palaeogenomic inquiry, an endeavour that our team is pursuing.

## DOMESTICATION OF ANIMALS OF TRANSPORT

The first animal that was domesticated not only to be a more convenient and abundant food source, but also to allow transportation of goods, was the donkey, followed by the horse and later the dromedary. The domestication pathway of these species has been classified as directed process (Zeder 2012). While the domestication process of the donkey has not been elucidated yet through palaeogenomics, probably because of poor DNA preservation in the areas of its habitat (North Africa, Southwest Asia), the analysis of the domestication process of the horse based on ancient genomes has already yielded great insights.

### **Domestication of the horse**

The origin and timing of horse domestication is one of the big questions of archaeology that could not be resolved solely through archaeological and archaeozoological research, despite the importance of the horse for post-Neolithic societies. An answer to the question of the timing of the horse domestication process has been found through a palaeogenetic study of Late Pleistocene and Holocene horse remains from Siberia, Central, southeast and eastern Europe, as well as the Iberian Peninsula (Ludwig *et al.* 2009). The authors analysed SNPs known from studies of present-day horses to be associated with genomic regions involved in the expression of the coat colour. The analysis showed that horses prior to *c.*5000 BP had the wildtype colour bay, while remains dated after *c.*5000 BP belonged to horses with a large number of various coat colours. Since coat colours constitute the most obvious phenotypic trait, it is thought that they were among the first characters subject to human-driven selection, which has also been

confirmed in the case of domestic goats. Moreover, this increase in coat colorations happened relatively rapidly, which would be rather unlikely if only natural selection were at work. Mutations associated with coat colour dilutions or spottings were found only later, around 2800 to 2600 years ago. The authors concluded that horse domestication started at least 5000 years ago. They also hypothesized that it happened in the Eurasian steppe, but the patchiness of the sample did not lend support to this hypothesis.

Interdisciplinary research at the archaeological site of Botai in the Central Asian steppe, dated to *c.*5000 BP combining archaeological, osteological, isotopic and chemical residue analyses proposed that the hunter-gatherers of the Eneolithic Botai culture herded, milked and harnessed horses (Outram *et al.* 2009). When the genomes of the corresponding horses were analysed and compared to those from later periods and present-day horses, however, it turned out that the Botai horses were related to the ancestors of Przewalski's horses, but not to horses dating between *c.*4000 years ago and today (Gaunitz *et al.* 2018). This unexpected result left the question of the origin of domesticated horses unanswered. Subsequent ancient DNA studies investigated other presumed centres of horse domestication, in particular the Iberian Peninsula (Uerpmann 1990, Warmuth *et al.* 2011) and Anatolia (Arbuckle 2012). The genomic information of horse remains from the 3rd-2nd millennium BCE on the Iberian Peninsula revealed a lineage that is different from Botai and Przewalski's horses, but also from the lineage leading to modern domesticates (Fages *et al.* 2019). Thus, the ancient wild horse population on the Iberian population that also harboured a particular mitochondrial lineage, was deemed to go extinct and was not the ancestral population of modern domestic horses.

Therefore, the search for the origin of domestic horses had to go on. To explore Anatolia as a potential domestication centre, we analysed post-cranial horse remains covering 9000 years from archaeological sites in Anatolia and Transcaucasia targeting mitochondrial lineages, Y chromosomal lineages, and nuclear markers associated with the coat colour (Guimaraes *et al.* 2020). This study detected two endemic mitochondrial lineages in Anatolian wild horses not present outside of this area at the time, one of which is now extinct. Moreover, while the mitochondrial diversity in horses prior to 2000 BCE was very low in this area, it quickly diversified afterwards (Figure 4). The same pattern was shown for the coat colour the diversification of which coincided with the mitochondrial lineages (Figure 4). The speed and magnitude of this diversification argues strongly for the introduction of domesticated horses from elsewhere that had been already selectively bred. Native Anatolian wild horses, however,

were shown to have been recruited into the domestic herd. The phylogeographic distribution of the mitochondrial lineages in prehistoric horse remains available to date hints to the Pontic-Caspian steppes as origin of these domestic horses and their introduction into Transcaucasia and Anatolia via the Caucasus, as also put forward by archaeological evidence (Anthony 2007). This hypothesis remains to be tested. We also identified the first mule in history from an Iron Age context at *c.*1200 BCE demonstrating the advanced breeding techniques of this early society (Guimaraes *et al.* 2020). This also shows that the properties of mules were considered superior to horses and donkeys, at least for certain aspects, since the breeding of sterile live stock is costly.

The analysis of ancient horse genomes covering *c.*5000 years revealed the existence of a so far unknown, presently extinct, horse lineage on the Iberian Peninsula that survived into the 7th century BCE in Central Spain, but was also found in a specimen from the 3rd millennium BCE in a Hungarian site that was a trading centre (Fages *et al.* 2019). This finding is suggestive of the role of horses in long distance exchange already in the Bell Beaker period. This endemic Iberian lineage contributed only marginally to the present-day domestic horse lineages, and its contribution was calculated to have occurred 34 – 44 000 years ago, revealing ancient gene flow between wild populations.

This study also unveiled for the first time the introduction between the 7th and 9th centuries CE of new domestic lineages in the South of Europe that were shown to be genetically linked to Sassanid Persian horses dated to the 4th-5th centuries CE. These are probably Arab imports and thus witnesses of the Early Muslim conquests of Europe. A similar population shift was observed in Central Asia and Mongolia.

A particularly interesting chapter of the history of horse domestication has been unravelled in the Eurasian steppes in the Scythian culture. The genomic data show that horses sacrificed and buried in Scythian elite burials were genetically and phenotypically diverse, hinting to the custom described by Herodotus of allied tribes endowing Scythian rulers with their horses (Librado *et al.* 2017). The genetic diversity of these horses, including the diversity of the Y chromosome, as well as the low inbreeding coefficients that were obtained in this study are suggestive of herding strategies that did not disrupt the natural herd structure and were not based on only a few stallions. Scythian horses shared more ancestry with a presently extinct Arctic horse lineage than do present-day domestic horses. This implies that the latter lost a

significant amount of these archaic components since the Iron Age.

The screening of the genomes of the Scythian horses for signatures of selection highlighted genes involved in milk production, mammary gland development, and the posterior pituitary gland which produces the neurophysical hormones oxytocin and vasopressin that are involved in lactation and social bonding. Moreover, enrichment in domestic horses for genes involved in cognitive, behavioural and certain morphological changes have been found that can be classified as belonging to the complex of genes involved in the above-mentioned domestication syndrome that is due to the loss of neural crest-derived cells.

Pre-domesticated wild horses have been shown to carry less deleterious mutations than extant domestic horses, suggesting that the domestication process caused a demographic collapse that reduced the efficacy of purifying selection (Schubert *et al.* 2014). Since this was not observed in the genomes of the Scythian horses, the authors concluded that deleterious mutations have accumulated in domestic horses only over the last 2300 years, thus later than predicted (Librado *et al.* 2017). Moreover, the observed loss of heterozygosity (loss of an entire gene and the surrounding chromosomal region) and of the Y chromosome diversity became particularly prominent during the last 200 years or so, while earlier societies maintained a higher level of genetic diversity (Fages *et al.* 2019). This relatively recent decay in effective population size, genetic diversity, and heterozygosity accompanied by an increase in the mutational load suggest severe reduction in breeding horse stocks within a short time resulting from changes in breeding strategies, probably linked to the invention of engine power.

## CONCLUSIONS

The access to the genomes of animals from prehistoric, protohistoric and historic periods has dramatically changed the field of animal domestication studies. Indeed, the analysis of a large number of ancient animal genomes or at least genome-wide information, has unveiled an unprecedented number of details about the domestication process, details that could not be obtained by more classical archaeological and archaeozoological approaches. It becomes more and more clear that this process was both different for each species, and a protracted process that involved accidental as well as human-mediated hybridization with the wild ancestors and selection for a few general and many species-specific phenotypic traits. As a rule of thumb, it

seems as if the first target of the taming process that preceded domestication was the behaviour in that the less aggressive and less fearful individuals were tamed. Once the animals were subject to reproduction control, other characters were selected, such as pigmentation, the most visible of domestic traits, as well as secondary product, such as meat and milk production, but also fertility, speed, stamina etc. The selection history is specific for each species and includes regional and temporal variations. In this way, the palaeogenomics of the domestication of animals also adds to the history of human societies.

In conclusion, the results obtained through palaeogenomic studies up to now, which are far from being accomplished, yield substantial insights into the complexity of the domestication process of several species and there is more to come.

#### ACKNOWLEDGMENTS

I would like to thank Thierry Grange and Caitlin Martin for their critical reading of the manuscript.

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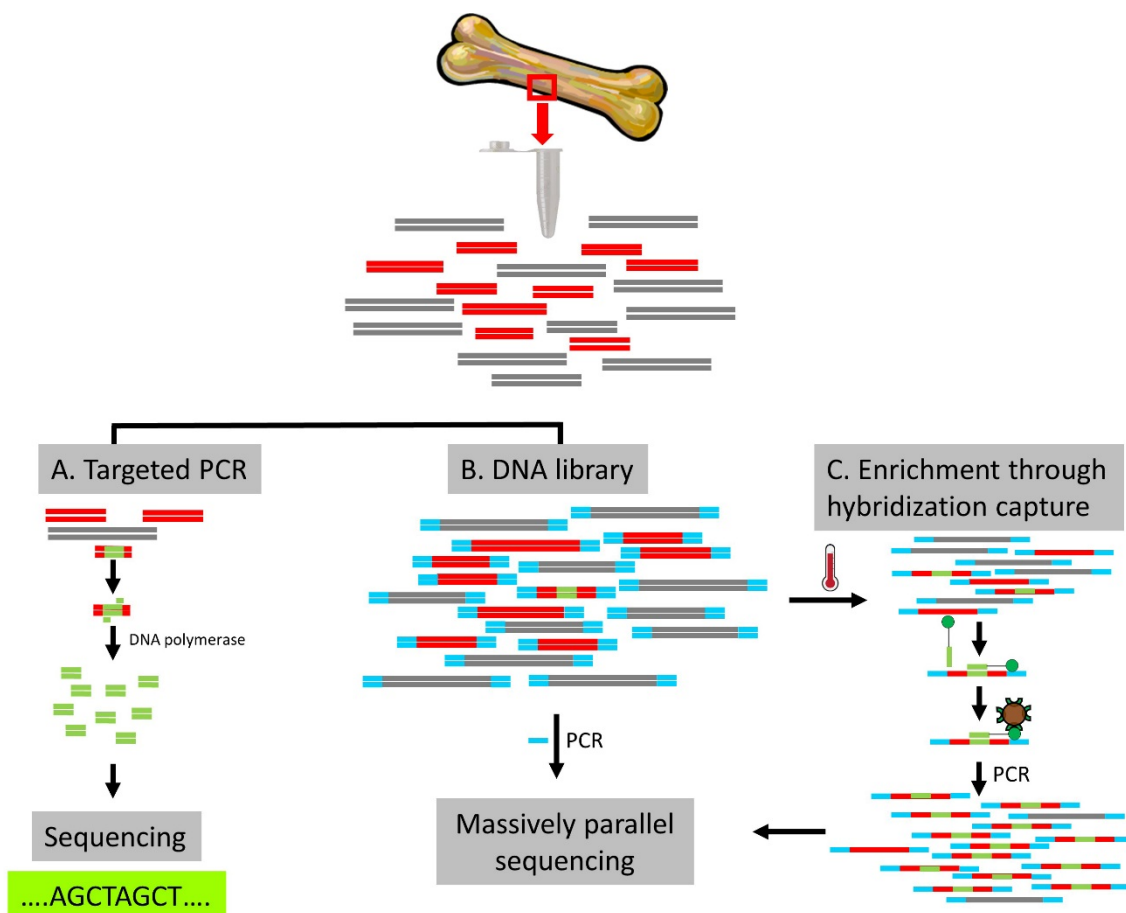
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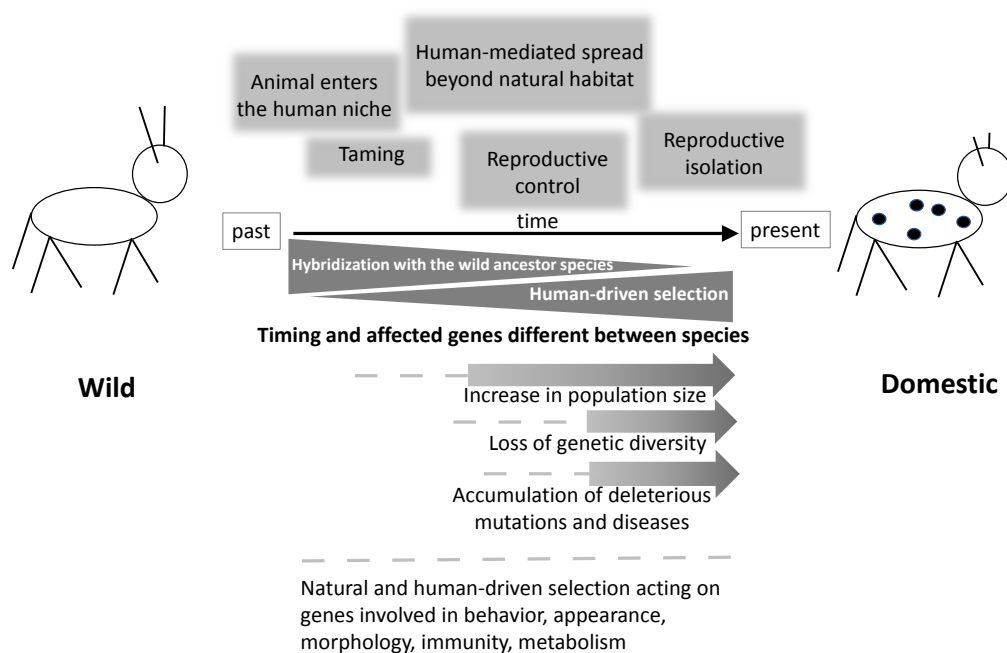
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## Figures

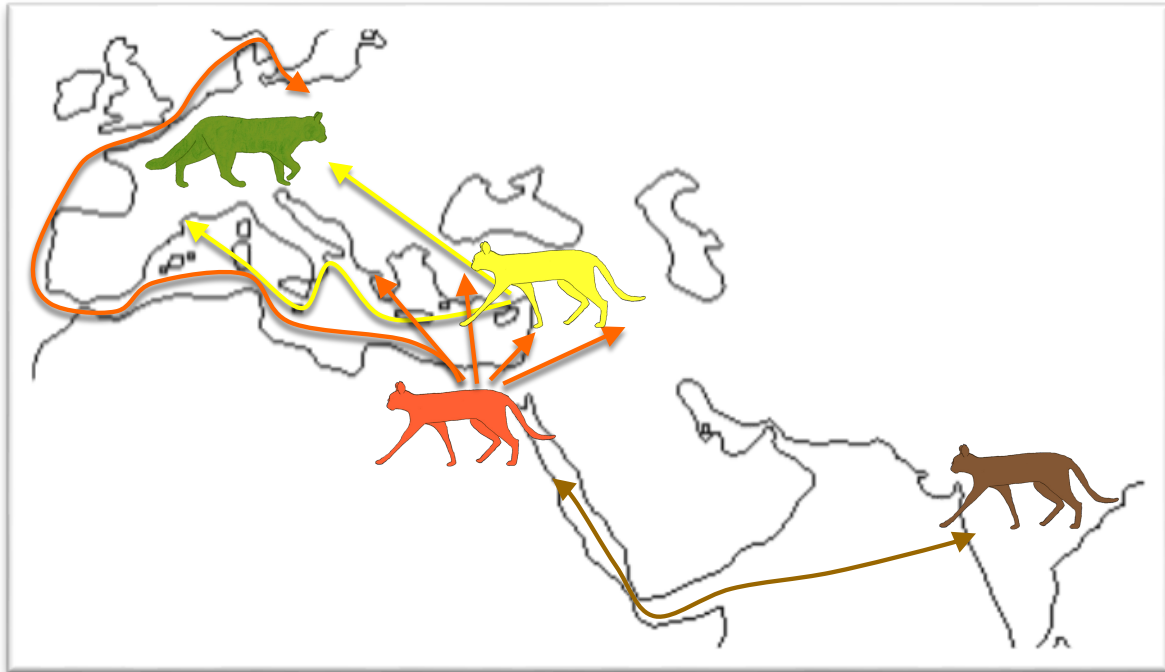


**Figure 1** Schematic representation of the three DNA analysis methods currently used. The extract from a bone is a heterogeneous mixture of DNA molecules of various origin: the animal to whom the bone belonged (red), bacteria and fungi from the soil that colonized the bone during burial (grey). A: Targeted PCR where only genomic regions of interest (green) are

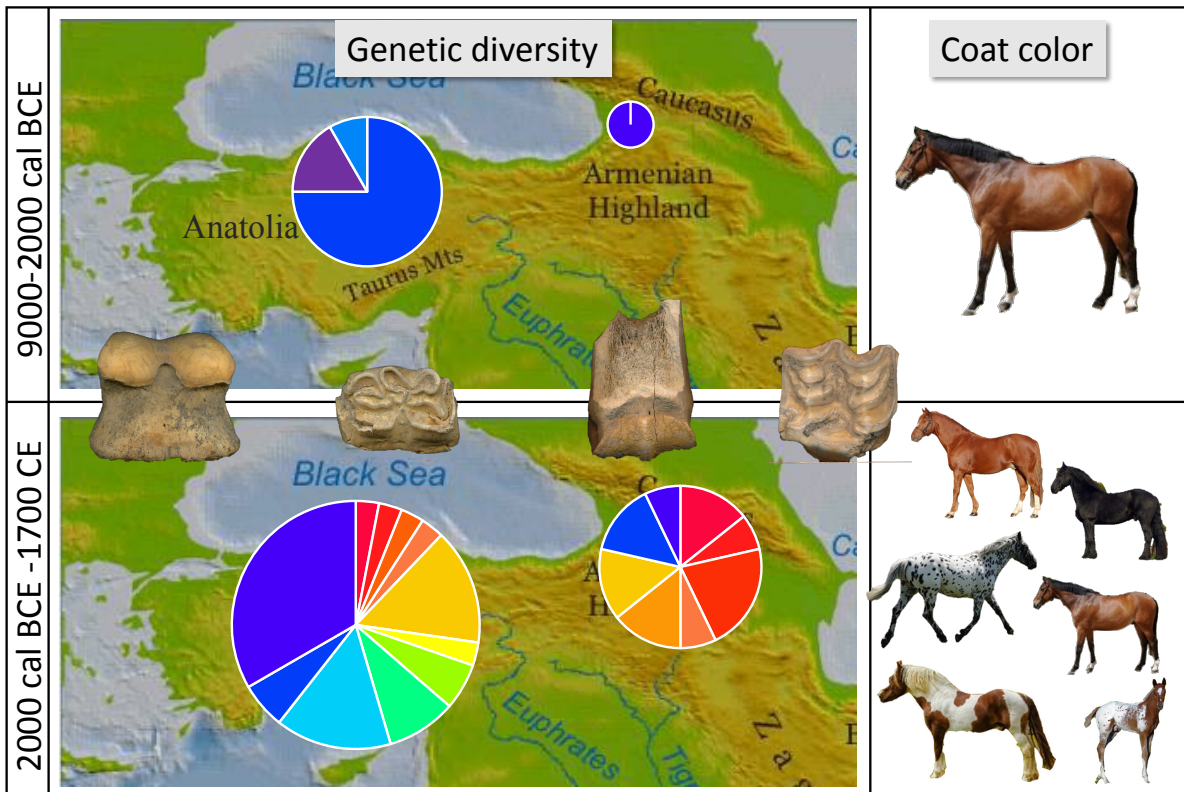
enzymatically multiplied with the help of small oligonucleotides of known DNA sequence (green) that bind to the complementary sequence on the extracted DNA molecule and act as primers for the enzyme DNA polymerase that synthesizes the complementary DNA strands. B: DNA libraries are constructed enzymatically by multiplying all DNA molecules in a DNA extract through small oligonucleotides of known sequence. The adapters (turquoise) that are ligated to the ends of the extracted DNA molecules and serve as primers for the DNA polymerase that will multiply all DNA molecules of the extract, endogenous DNA (red) and environmental DNA (grey). After multiplication through PCR, all the DNA molecules will be sequenced through a procedure that sequences the DNA molecules massively in parallel, the so-called ‘next generation sequencing’. C. In order to enrich for certain target DNA molecules, DNA baits (probes) are produced that carry a DNA sequence complementary to the targeted DNA molecules. These are bound to magnetic particles that are captured through a magnet. The captured molecules are enriched and sequenced subsequently to their PCR amplification.



**Figure 2** Schematic representation of the mechanisms shaping the genomes of animals through the domestication processes that are discussed in the text. The timing of the various processes varied from species to species and so do the genes involved.



**Figure 3** Spread of the cat as deduced from the mitochondrial lineages (haplotypes) identified in archaeological remains (Ottoni et al. 2017). While in archaeological sites in Western Europe prior to the Iron Age only the maternal lineage of the European wildcat *Felis silvestris silvestris* was found (green), remains in early Neolithic sites in Anatolia carried a mitochondrial haplotype of the Near Eastern cat *Felis silvestris lybica* (yellow). This haplotype appeared in early Neolithic sites in Southeast Europe testifying to the human-mediated translocation of cats, which suggests some taming process having occurred. Cats in Egypt during the Ptolemaic period carried the Anatolian and yet another haplotype (orange). This haplotype was found at high frequency a few centuries later in Roman sites in Anatolia and then in Europe, and even in Viking sites at the Baltic Sea. In a Roman port in Egypt, with vivid trading connections to India, some cats were carriers of the lineage of the Southern Asian wildcat *F.s. ornata* (brown).



**Figure 4** Evolution of mitochondrial haplotypes and genetic markers associated with the coat colour in horse remains in Anatolia and the Southern Caucasus from 9000 cal BCE to 1700 CE (Guimaraes et al. 2020). While only four mitochondrial haplotypes were found prior to 2000 cal BCE, two of which have never been identified elsewhere, the diversity increased dramatically after 2000 cal BCE. The same dynamics were found to have occurred for the coat colour markers. Only the wildtype bay colour was identified prior to 2000 cal BCE, and a large variety of coat colours afterwards. Photographs taken by B.S. Arbuckle of a few of the analyzed bone and teeth remains, and photographs of present-day horses showing the coat colours identified in this study (Source: Institut du cheval et de l'équitation ifce, France).