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Thousands of Years of Relationship between Man and Dog Revealed by Genomics

Morgane OLLIVIER
ECOBIO, Université de Rennes, France

The dog was the first animal to be domesticated by humans, at least 25,000 years ago. Its domestication was made possible by the wide and abundant distribution of its wild ancestor, the wolf, in the northern hemisphere, where human societies were evolving at the end of the Paleolithic era. The wolf's history is therefore atypical of other domesticated species, as its domestication began well before the Neolithic transition. It is not easy to pinpoint a precise date for domestication.

It is important to remember that domestication is a gradual process, and so the dog/wolf distinction is only possible when the characteristics of domestication are visible. The first dogs were probably morphologically very similar to wolves, and so cannot easily be distinguished from one another. Wolves also had a very wide distribution (the entire northern hemisphere), making it all the more difficult to classify remains solely on the basis of geography. Canid fossils are also rare, and consequently the archive is both temporally and geographically fragmented. In this chapter, we will review the main lines of research carried out over the last 20 years, which have shed light on the evolutionary history of the dog and its relationship with man.

Genetics of Domestications,
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1.1. The history of dog domestication, the subject of much debate

1.1.1. *The dog: domesticated since the Paleolithic period*

The appearance of dogs in the fossil record is recent, and current debates center on whether dogs first appeared in the late Paleolithic or early Neolithic periods. On archaeological grounds, the divergence between dog and wolf is thought to have occurred between 16,000 and 11,000 years BP, a time interval that corresponds to the age of the first undisputed ancient dog remains found in both Europe and Asia at numerous remote archaeological sites (Vigne 2005). Earlier dates have been proposed on the basis of remains initially assumed to be dogs. In Europe, these include remains from the sites of Předmostí in the Czech Republic, estimated at 27,000 years BP, and Goyet in Belgium, estimated at 36,000 years. In Asia, remains found in the Altai Mountains in Russia date back some 33,000 years. However, recent work has cast doubt on these conclusions, demonstrating that the morphology of the specimens studied was within the range of morphological variation of late Pleistocene wolves. For example, fossil Belgian canids, including the Goyet dog, form a sister group to all contemporary dogs and wolves, suggesting that they represent either a failed domestication event, or a morphologically distinct form of wolf.

In 2015, the genome of a 35,000-year-old wolf from the Taimyr Peninsula in northern Siberia was sequenced. By comparing this genome with that of the modern wolf and that of the present-day dog and based on an estimate of the mutation rate between these genomes, it was concluded that the ancestors of dogs were separated from present-day wolves before the Last Glacial Maximum and the Taymir wolf (Figure 1.1) belonged to a population that had already diverged from the common ancestor of modern wolves and dogs. Dogs are probably descended from extinct wolf populations that diverged from the ancestors of extant wolves around 27,000–40,000 years ago (Skoglund 2015). Present-day gray wolves would not be their direct ancestors, and it is likely that the modern-day dogs, which are closest to these wolves, are the result of multiple hybridizations (Frantz et al. 2016). The disappearance of these populations could be explained by the decline in wolf populations during the Last Glacial Maximum, which then led to the repopulation, expansion and replacement of ancestral populations by current populations. The wolf population from which dogs most likely descend were large northern wolves, and none of the current wolf lineages from the hypothetical regions of domestication appear to be the original source population (Skoglund et al. 2015). These studies therefore suggest that dogs probably originated in Eurasia as early as 33,000 years ago, an earlier date than their first record in the archaeological record, and that their evolutionary history is complex along a long phenotypic continuum.

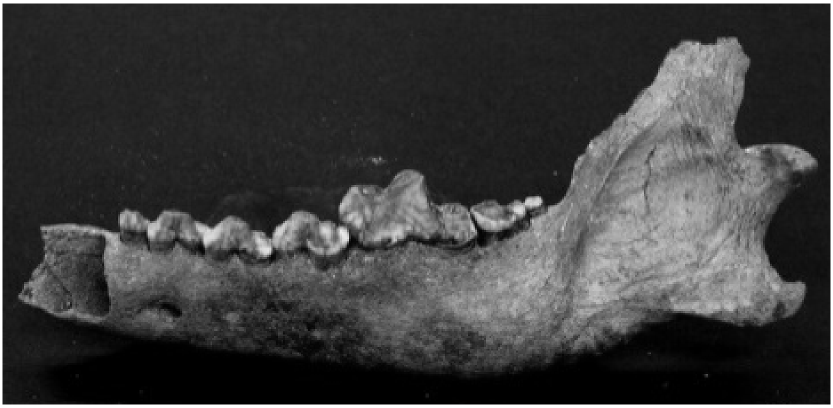

Figure 1.1. *Taymir wolf mandible fragment (source: Reuters). For a color version of this figure, see www.iste.co.uk/pelletier/genetics.zip*

1.1.2. *The process behind dog domestication*

Domestication leads to the appropriation and control by a human society of an animal or plant sub-population for the production of a service or commodity. It involves an evolutionary process that documents two aspects of evolution: speciation and adaptation. In the domestication process, these two biological mechanisms consist, on the one hand, of a relative break in gene flow between the original population and the domesticated population and, on the other hand, of a modification in selective pressures. Based on ethnographic research suggesting that domestic animal husbandry was not unusual among hunter-gatherer groups worldwide, Francis Galton suggested that dogs had been domesticated following the capture and rearing of cubs in human camps. Later, Belyaev (1969) showed that animal domestication could have begun in the absence of deliberate human design and action. In 2011, Vigne (2011) proposed a multi-stage model characterized by a gradually intensifying relationship between humans and animals. In this perspective, animal domestication proceeded along a continuum from anthropophilia to commensalism, control in the wild, control of animals in captivity, extensive breeding, intensive breeding and, finally, pets. While Zeder (2012) has also acknowledged this model’s step-by-step approach, she describes three distinct paths that animals may have followed in a domestic relationship with humans: a commensal path, a prey path and a directed path.

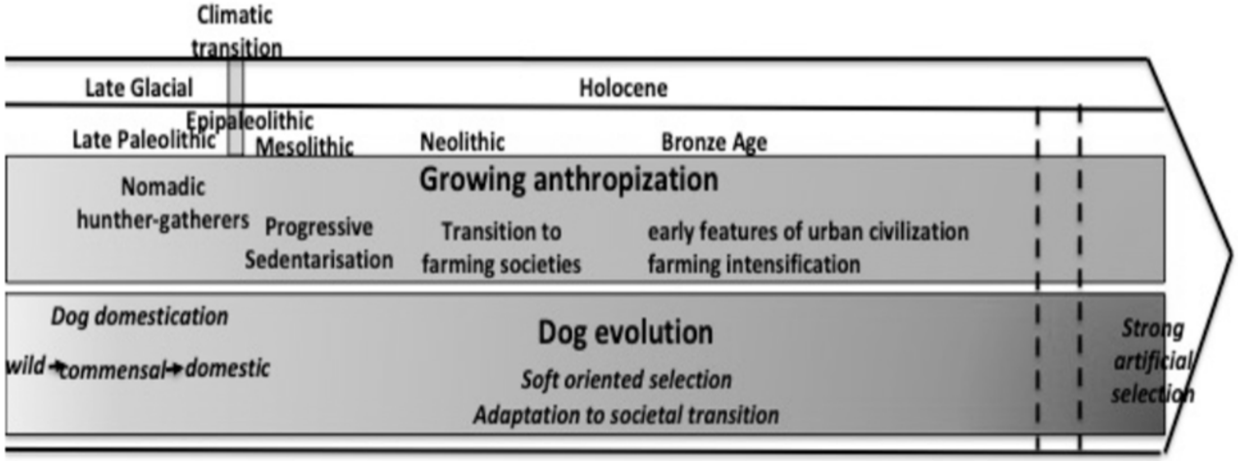

Figure 1.2. *Schematic representation of the chronology of human societal change and the history of the dog in Western Europe (source: M. Ollivier). For a color version of this figure, see www.iste.co.uk/pelletier/genetics.zip*

In the case of the dog, it is assumed that the domestication process first took the form of commensalism/mutualism (Vigne 2011) between wolves and humans at the end of the Paleolithic era (Vigne 2005). The commensal pathway does not begin with intentional human action to bring wild animals (juvenile or otherwise) into their camps. Instead, different wild animal populations would have been attracted by elements of the human niche, including human food waste.

The individuals most able to take advantage of the resources associated with human camps would have been the more docile, less aggressive individuals. A recent study supported this view, proposing a hypothesis based on the sharing of resources between humans and wolves. Humans are not fully adapted to a carnivore diet, as their meat consumption is limited by the liver's ability to remove the ammonia produced by protein digestion.

Unlike humans, wolves can thrive on lean meat for months on end. Yet the study shows that all Pleistocene archaeological sites where dog remains have been found originate from areas similar to subarctic and arctic environments. Calculations show that during harsh winters, when game was lean and lacking in fat, late Pleistocene hunter-gatherers in Eurasia would have benefited from a surplus of animal protein that could have been shared with early dogs. This study explains how competition may have contributed to the initial phase of domestication.

Thus, the relationship between man and dog probably involved a gradual taming of the wolf linked to a cultural process (Larson and Burger 2013), particularly in Europe, where it is likely that such interactions began over 30,000 years ago. After the first phase of domestication, dogs would have become more docile, being used in a multitude of ways, such as hunting companions, beasts of burden, guards, and would have undergone many evolutionary changes similar to those of humans.

1.1.3. *The number and location of domestication events: contextualization and the contribution of archaeological and genomic data*

Gray wolves were distributed throughout the northern hemisphere, and it is assumed that the wolf populations that gave rise to dogs, in whole or in part, are certainly extinct. Furthermore, the human populations that domesticated wolves were mobile hunter-gatherers. For these reasons, it has long been difficult to establish where dogs were domesticated, or even whether several wolf populations were domesticated independently. Several morphological groups can also be distinguished among Upper Paleolithic dogs: large, robust canids are observed in northeastern and central Europe, while medium-sized dogs with strong allometric differences are described in the Middle East. Lastly, small-sized individuals are found in southwestern Europe. This morphological diversity, as well as the temporal concordance of dog samples found in both Europe and Asia, with the wide distribution of wild ancestors and Upper Paleolithic dogs, has been interpreted by archaeozoologists since the 19th century as a strong argument for multiple, independent domestications.

Genetic studies of the diversity of today's dog breeds, on the other hand, have led to contradictory and sometimes opposing conclusions. Analysis of the ***mitochondrial DNA*** of 162 wolves from 27 locations worldwide, and 140 domestic dogs representing 67 breeds, shows that most dog sequences belong to a single group in which no wolf sequences are found. However, in three other groups, both dog and wolf mitochondrial sequences are found, which the authors interpret as an initial domestication event, followed by repeated cycles of hybridization and selection for canine phenotypic variation. Repeated genetic exchanges between dog and wolf populations may have been a significant source of variation for artificial breed selection. Analysis of a 582-base-pair region of mitochondrial DNA from 654 domestic dogs from Europe, Asia, Africa and Arctic America, as well as 38 Eurasian wolves, shows that haplogroup A1 is dominant over the other five (B, C, D, E and F), and that dogs with a Southeast Asian origin show greater genetic diversity. This DNA region was also analyzed in 1543 Old World dogs, including modern breeds of known geographical origin, as well as breeding dogs of indigenous ancestry, which are more relevant for unravelling the origins of the dog than modern breeds. The results point in the same direction, showing greater genetic diversity in Asia, with Western Eurasian ***haplotypes*** all attributed to an East Asian haplotype. These studies thus proposed East Asia as the main and only center of domestication, based on comparisons of genetic diversity between regions, hypothesizing that centers of origin contain greater genetic variation. A new interpretation was subsequently given to this observation by some authors, who suggested that it reflected a replacement of native European canine ancestry by more recent lines of Southeast Asian immigrants. This replacement may have been accompanied by a general geographical expansion of East Asian dog populations or, alternatively, explained by human transportation in connection with trade.

Other studies, again based on modern DNA, suggest, on the contrary, and in line with archaeological evidence, that several centers of domestication existed in Eurasia. For example, mitochondrial DNA diversity in African village dogs is comparable to that of East Asian dogs. This discovery was used to question the Southeast Asian origin of the dogs. Subsequent analysis of 48,036 nuclear genome ***SNP*** positions in 912 modern dog breeds, 225 wolves and 60 coyotes, demonstrates that Middle Eastern wolves contribute a significant amount of genomic diversity to modern dog breeds, suggesting a Middle Eastern contribution to the history of dog domestication.

Various observations may explain why it is so difficult to unravel the origins of dogs from current genetic data: (i) most dog breeds have very recent origins, with a history going back less than 150 years; (ii) dog populations have undergone numerous episodes of diversification, hybridization and homogenization, reducing the resolving power of genetic data from modern breeds; (iii) a combination of ***introgressions*** and bottlenecks between dog and wolf populations, well after the domestication process, may have blurred modern genomic signatures and reduced our ability to make a distinction between the initial domestication process(es) and post-domestication ***gene flow***; (iv) the wolf population(s) that gave rise to modern dogs may be extinct, making it difficult to detect the initial process using studies based on modern data. The ***paleogenomic*** approach therefore appears to be the most effective tool for tracing the history of dog domestication.

1.2. Paleogenomics: an essential tool for understanding the evolutionary history of the dog

Understanding the origins and early history of dogs has been complicated by bottlenecks, expansions, extinctions as well as local replacements and gene flows between wolves and dogs. Large-scale systematic research and analysis of ancient wolf and dog genomes across space and time is therefore needed to accurately reconstruct the evolutionary history of the first domestic animal.

1.2.1. *Eurasian origins and diffusion*

In 2013, the first mitochondrial genomes of ancient dogs and wolves were published and began to provide answers. The results show that dogs and wolves do not form two distinct ***monophyletic groups***. Each of the ***clades*** grouping modern dogs of haplogroups A, C and D have an ancient lineage as a sister group, grouping ancient European dogs and wolves. It was thus demonstrated that dogs have a European origin and have been descended from a line of wolves for over 20,000 years, a line that is now extinct. Following this, the analysis of over 99 mitochondrial sequences of Upper Paleolithicto Bronze Age individuals from 29 archaeological sites in Eurasia, and the complete genome of the Newgrange dog (late Neolithic, ~ 4,800 ago) in Ireland suggested that dogs, in both Eastern and Western Eurasia, were independently domesticated in the Upper Paleolithic from several wolf populations (Frantz et al. 2016). Later, during the Neolithic and early Bronze Age, Asian dog lineages spread westwards, leading to a profound change in the European dog population (Ollivier et al. 2018). Two types of diffusion seem to have taken place: one linked to the Neolithic transition, which may have originated in the Middle East, and a later one from Asia to Europe at the end of the Neolithic/Chalcolithic period, probably via the Pontic steppes and accompanied by other elements such as millet, the domestic horse and early metallurgy. Thus, human movements and cultural diffusion have profoundly shaped the lineage of dogs. Lastly, a very recent study shows that this scenario could be even more complex. By sequencing 27 ancient dog genomes from across Eurasia, Bergström et al. (2020) confirm that all dogs derive from a lineage of wolves that are distinct from present-day wolves and also show, as suggested by archaeozoological data, that five major lineages were already present and diversified 11,000 years ago, as early as the Paleolithic period. The fate of each of these lineages is therefore linked to, and even mirrors, migrations and the history of human populations. By studying the evolutionary history of the dog, we can retrace the history of mankind.

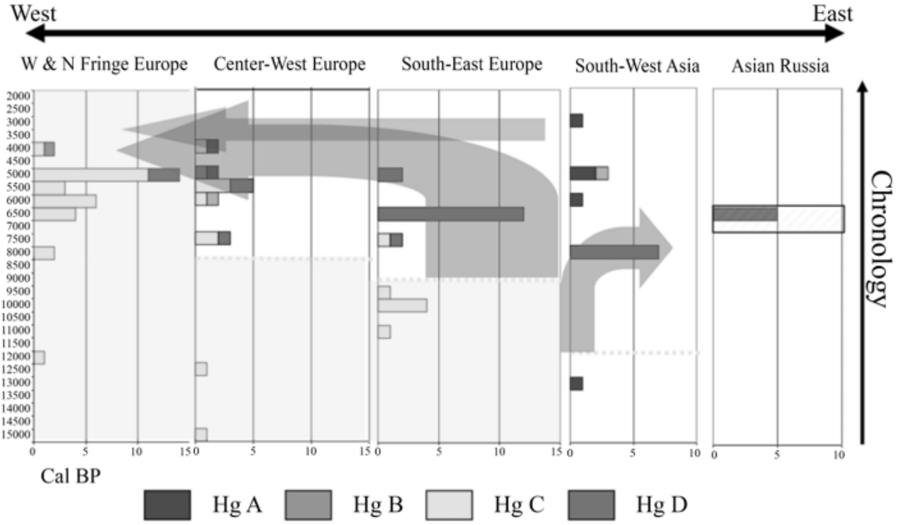

Figure 1.3. *Dog population movements in Europe between the Paleolithicera and the Bronze Age, inferred by mitochondrial data (Hg: haplogroup; BP: before present) (source: adapted from Ollivier et al. (2018)). For a color version of this figure, see www.iste.co.uk/pelletier/genetics.zip*

1.2.2. *The uniqueness of the Iberian Peninsula*

The Iberian Peninsula, like Italy and the Balkans, was a refuge during the Ice Age, preserving a unique diversity. The earliest evidence of dogs dates from

7,900 to 7,600 years ago in archaeological contexts accumulated by hunter-gatherer societies, a few centuries before the arrival of the first Neolithic communities.

By analyzing the remains of five Mesolithic dogs and 15 samples of dogs dating from the Roman period, collected from archaeological sites in Portugal, the authors show a high frequency of mitochondrial haplogroup A, the majority in present-day dogs. Eighty percent of the Mesolithic sequences from Portugal belong to this haplogroup. This result provides evidence that haplogroup A was present in Europe before the Neolithic period. Until now, haplogroup C was considered the most common clade in Europe before the Neolithic era (Frantz et al. 2016). Haplogroup A, observed at higher frequencies in the Middle East and Asia, had been considered to have been introduced to Europe after the arrival of Neolithic farmers, perhaps in the Bronze Age (Ollivier et al. 2018). These results suggest, in contrast, that this haplogroup may have been present in European refuges prior to Neolithic influence. These Mesolithic dogs could be attributed to local domestication(s), which is a scenario corroborated by mitochondrial data from Paleolithic Iberian wolves. Among the samples dating from the Roman period, 12 from Portugal, Spain and Morocco belong to clade A, and three from Spain belong to clade D. So far, this is the oldest evidence of clade D in the Iberian Peninsula. This result supports the idea that there may have been significant consolidation of dog breeds from divergent genetic lineages during this period. According to these data, local breeding involving dogs of clade A and clade D in the Iberian Peninsula was continuous for at least 1,600 years. In addition, the sharing of lineages between Spanish and North African dogs may suggest genetic flow. Dogs could have been easily transported between these regions by humans following shipping trade routes.

1.2.3. *Origins of the dog in America*

The oldest dog records in North America are found in Alaska and Illinois, dating back 9,020 ± 85, 8,820 ± 30 and 8,840 ± 80 years, respectively (Perri et al. 2019; da Silva Coelho et al. 2021). The origins and fate of dog populations in the Americas, prior to contact with European and African peoples, have been the subject of recent study involving comparisons of ancient and modern dog genomes. Comparative genomic analyses demonstrated that the first American dogs had no ancestry with American wolves. On the contrary, these Pre-Contact Dogs (PCDs) represent a specific lineage that may have migrated from Northeast Asia across the Beringian steppe alongside humans, over 10,000 years ago. These analyses also showed that PCD populations were subsequently almost completely replaced by European dogs during the large-scale colonization of North and South America over the last 500 years. The introduction of infectious diseases during this recent colonization probably played a major role in the replacement of PCDs by European dogs.

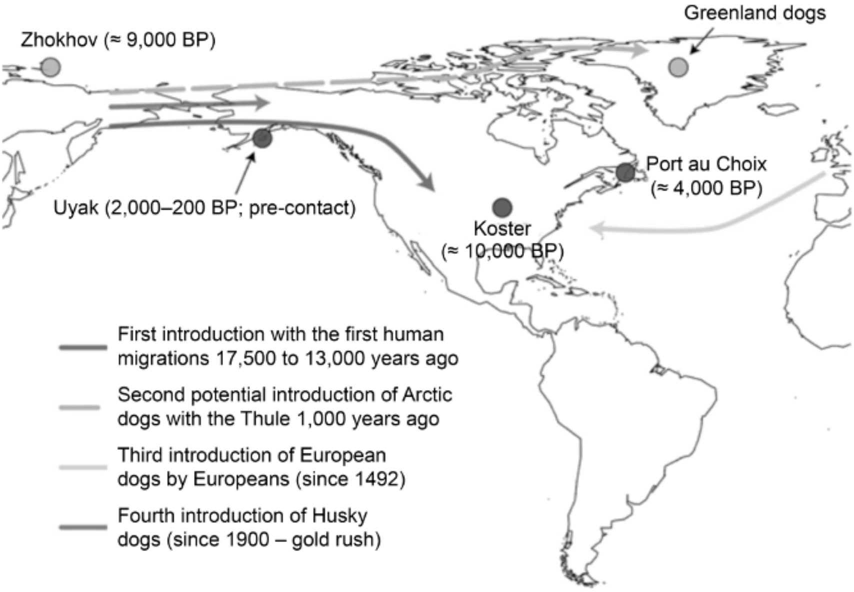

Figure 1.4. *Multiple dog introductions in North America (BP: before present) (source: M. Ollivier, adapted from Leathoblair et al. (2019)). For a color version of this figure, see www.iste.co.uk/pelletier/genetics.zip*

The introduction of dogs into South America remains to be clarified, but most probably follows the introduction of dogs into North America via Beringia, after the arrival of humans in South America. Indeed, the oldest dog remains found in America are in the north of the continent and post-date the arrival of humans in the far south of the continent 11,000 years ago. Analysis of the mitochondrial genomes of North American dogs has shown that pre-Columbian dogs belong to a monophyletic group (haplogroup A), having diverged 14,600 years ago, sharing a common ancestor with East Siberian dogs at least 16,700 years ago. The most likely hypothesis is that they migrated with human populations from North to South America. Mitochondrial DNA analysis of several pre-Columbian Mexican dog specimens (Manin et al. 2018) supports this hypothesis. Four haplotypes belonging to haplogroup A were identified. Three of these haplotypes are common to dogs of European and American origin. These dogs, probably originating from Eurasian populations that colonized North America, reached Central and South America, where they remained isolated for several thousand years. The last haplotype, however, is close to a sequence that is only shared by modern-day Chihuahua dogs, which is thought to have originated in Mexico. This proximity suggests that it is a characteristic haplotype of pre-Columbian dogs, resulting from the genetic divergence of these local populations over the thousands of years following their introduction. Lastly, a recent ***phylogenetic*** analysis of ancient mitochondrial genomes from South American dogs confirms that pre-Columbian and modern South American dogs share a common ancestry and belong to haplogroup A. In this study, the authors also show that present-day American dogs and pre-Columbian dogs show a high degree of genetic divergence, supporting the hypothesis that pre-Columbian dogs were almost completely replaced by European dog populations after the arrival of Christopher Columbus.

1.3. From commensalism to modern breeds: identifying the genomic foundations behind the intensification of the human–dog relationship

Domestication involves an evolutionary process that documents two aspects of evolution: speciation and adaptation. In the domestication process, these two biological mechanisms consist, on the one hand, of a relative break in gene flow between the original population and the domesticated one, and, on the other hand, of a modification in selective pressures. Some studies have highlighted the importance of progressive intensification of the relationship between man and animals, leading, in the case of the dog, to a continuum from commensalism, to captive animal control, to breeding and, ultimately, to pets (Vigne 2011; Zeder 2012). Isolating, taming, controlling and moving animals into an anthropogenic ecosystem has morphological, physiological and behavioral consequences that can be identified as a sequence of common traits selected along this trajectory from wild to domesticated (Belyaev 1969; Arbuckle 2005, pp. 18–33; Trut et al. 2009). During the process of domestication, a relaxation of natural selective pressures allowed the persistence of mutations linked to these new traits, resulting in an ever-increasing divergence between the genomes of domesticated animals and those of their wild ancestors. Unlike other domesticated animals, domesticated canids have also been subject to progressive human impact on their environment, as a result of changes in socioeconomic systems and human subsistence patterns. Such shifts in cultural contexts, as well as the resulting new selective pressures, could also have had an impact on the evolution of the dog genome, by leveraging changes already in place, or enabling the emergence of new changes. In general, however, it is difficult to identify regions of the genome that are unambiguously associated with early stages of domestication, and then implicated in current phenotypic diversity. This is because (i) there may only be a very limited number of regions involved in this process, a phenomenon particularly highlighted in dogs; (ii) some traits (such as behavior) have a complex genetic basis such as multiple gene inheritance and epistatic relationships[1], or are linked to regulation of gene expression; (iii) the regions identified may be large, without it being possible to accurately identify the genes or variants involved; (iv) studies are costly and time consuming (Larson and Burger 2013; Larson et al. 2014). Despite this, the change in scale of analysis (from the candidate gene to the genome) in recent years has made it possible to study the domestication process at high resolution in order to determine how it has shaped modern domesticated animals. However, understanding the basis and genetic architecture of the domestication process remains a challenge.

1.3.1. *Selection and domestication markers*

The mitochondrial and nuclear genomes of the dog possess an overabundance of ***non-synonymous mutations*** when compared to wolves and coyotes, which can be explained by a relaxation of selective pressures after domestication. Models suggest that once animals are freed from the selective pressures associated with life in the wild, they accumulate non-synonymous mutations under a relaxed selection regime, some of which led to new phenotypes that humans might have preferentially selected (consciously or unconsciously), while nature would have actively eliminated them. This increase in the number of non-synonymous mutations is observed in all modern dog breeds, suggesting the presence of small populations during and after domestication.

The detection of genomic ***loci*** with ***selective sweep*** signatures allows us to identify candidate regions that may have undergone rapid micro-evolution during, and shortly after, domestication. Many of the regions identified concern lipid or starch metabolism, immunity, behavior, brain function and pigmentation. These findings are consistent with the shared history of humans and domestic dogs. For example, with the development of agriculture, metabolic mechanisms to digest increasing proportions of starch from cereal crops were selected. Thus, among the candidate genes for the domestication process, the *MGAM* gene has been identified as being under positive selection. This gene is involved in the final stages of starch digestion. Similarly, one of the strongest selection signals between dogs and wolves is centered around *CCRN4L*, a gene that controls lipid metabolism through its interaction with *PPAR-Υ*. The selection of this candidate gene is consistent with the change in lipid content in the diet of the earliest domestic dogs. When dogs and humans began to hunt together, the number of prey caught probably increased

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[1] Functional interactions between different genes, like when an allele of gene A masks the effect of an allele of gene B.

relative to the number caught by wolves alone, and with it, the amount of lipid consumed by early dogs. A unique dietary selective pressure may thus have resulted from both the amount of lipids consumed and the composition of the tissues made available to these dogs after humans had removed the best parts of the carcass. The dogs' behavior was also markedly altered from that of the wolves in terms of docility and canine understanding of human facial expressions and actions. Thus, all comparisons of dog and wolf genomes revealed evidence of selection on candidate neurobehavioral genes. For example, *MBP*, a schizophrenia-related gene responsible for myelin sheath formation, and *SH3GL2*, a gene involved in synaptic vesicle formation, were found in regions identified as being under positive selection in dogs (Axelsson et al. 2013).

1.3.2. *Genetic variation and adaptation to a starch-rich diet*

In western Eurasia, the Neolithic transition took place between 11,500 and 6,000 years ago, with the transition from hunting and gathering to agriculture. By this time, the dog, which was domesticated during the Upper Paleolithic, had already been accompanying humans for several millennia. The study of the impact of changes in diet, which was brought about by these human societies, on the physiological changes of the first domesticated canids and the transformations in their genomes that gave rise to today's canine genotypes and phenotypes, is of crucial importance.

A comparison of current dog and wolf genomes has identified genomic regions potentially affected by this process. In particular, a series of duplications of the gene coding for pancreatic amylase (*Amy2B*) appears to have been selected, leading to an increase in the number of copies of this gene in modern dogs. An increased number of *Amy2B* copies is associated with higher amylase activity, enabling improved starch digestion capacity (Axelsson et al. 2013). While the number of *Amy2B* copies widely varies in dogs (4–34 copies), the number is much lower (two to eight copies) in wolves, with 60% of wolves carrying only two copies of the gene. This suggests that dogs are better adapted to a starch-rich diet than wolves. Present-day canids show three patterns of variation in the number of *Amy2B* copies: (i) 60% of wolves and most dingoes carry two copies of the gene; (ii) dogs and wolves carrying between two and eight copies of *Amy2B*; (iii) dogs carrying more than eight copies of *Amy2B*. However, the question of a link between the increase in the number of *Amy2B* copies in dogs and the Neolithic transition remains unanswered with these data, because while this increase could have provided a strong adaptive advantage in an agricultural context, we cannot rule out that it occurred much later, following the more recent selection of specialized lineages.

Paleogenetics offers a unique opportunity to shed light on this question, through studying the genetic landscape of the varying number of *Amy2B* copies in ancient canid populations. The study of the number of copies of the *Amy2B* gene in 88 ancient dogs from various archaeological sites across Eurasia yielded results for 13 of them. Four of the ancient dogs – from Romania (6,500 years ago), Turkmenistan and France (4,000–5,000 years ago) – possessed more than eight copies of *Amy2B*. The hypothesis of a modern origin explaining the increase in the number of copies is ruled out. The earliest expansion of the *Amy2B* gene number could be dated to 7,000 years ago (Neolithic era) and probably constituted an important adaptive advantage for dogs that were able to feed on the waste or remains of human meals (Ollivier et al. 2016). Interestingly, five of the samples appear to only possess two copies, which is a situation that is almost nonexistent in present-day dogs. Dog populations with low *Amy2B* copy numbers are currently associated with nonagricultural populations (Arent 2016). This pattern supports the hypothesis of an ancient expansion of *Amy2B* associated with the Neolithic period. Ultimately, it is worth noting that individuals presenting the three profiles (two copies, three to eight copies, more than eight copies) can be found on the same archaeological sites, which shows that in the Neolithic era, the expansion of the *Amy2B* gene was not yet fixed in dog populations associated with Neolithic agricultural societies. These results have recently been corroborated (Bergstrom et al. 2020) by showing that several Neolithic dogs (Iran: 5,800 years old; Spain: 6,200 years old) do indeed possess a high number of copies of the *Amy2B* gene. This increase, which was variable among dog populations associated with the first farmers, was widespread and fixed for a few thousand years with the regular use of starch-rich agricultural products.

1.3.3. *The evolution of coat color*

Analysis of gene sequences encoding phenotypic traits in ancient dogs can provide information on their phenotype, on the diversity of the trait in ancient populations, and may even be an indicator of the impact of domestication. Changes in coat color is often considered one of the traits that can vary from earliest stages of domestication (Belyaev et al. 1969; Trut et al. 2009). Genetic landscape analysis of coat color variation has shown the presence of such variations in ancient populations of Eurasian dogs and wolves. The isolation of DNA fragments of two genes controlling coat color, *Mc1r* (melanocortin 1 receptor) and *CBD103* (canine-β-defensin), from 14 different archaeological sites spanning a period from the late Upper Paleolithic to the Bronze Age, detected a new variant (*R301C*) of the melanocortin 1 receptor, highlighting the presence of the melanistic beta-defensin mutation (*CDB103-K* locus) in the genomes of several ancient dogs. The results show that the dominant

K^B (*CBD103*) allele, which is responsible for melanism, and the *R301C* (*Mc1r*) variant, which can cause light coat coloration, have been present since the beginning of the ***Holocene*** era in Eurasia, over 10,000 years ago. These results underline the genetic diversity of prehistoric dogs, inherited not only from the wild pool from which the dogs originated (*R301C* variant), whose variability remains largely unknown, but also from mutations most likely linked to the relaxation of natural selection (such as the K^B variant).

1.3.4. *The question of human–dog co-evolution*

Multiple studies aimed at understanding the origin and spread of dogs around the world have highlighted that dogs have accompanied humans throughout their migrations (Ollivier et al. 2018; Bergström et al. 2020). Joint analysis of ancient human and canine genomes has also revealed that part of the history of canine populations mirrors that of humans (Bergström et al. 2020). Thus, the diversification of at least five lineages of canine ancestry, at the beginning of the Holocene era, was probably the result of a dynamic reflecting the way dogs migrated alongside humans. However, this phenomenon is not a generality and, in many cases, the geographical distributions of dogs and humans do not always correspond, revealing a complex history of different canine lineages. This suggests that humans have also migrated without dogs, that dogs have moved between human groups or, like other domestic animals, dogs may have been the object of commercial, cultural or economic exchanges.

An example of bio-cultural co-evolution is the increase in the number of copies of the *AMY2B* gene involved in starch digestion, linked to dietary adaptations in dogs during the agricultural transition (Axelsson et al. 2013; Ollivier et al. 2016; Bergström et al. 2020). The homologous[2] human *AMY1* gene has probably undergone adaptive evolution, although this has not been clearly linked to agriculture. It is therefore reasonable to hypothesize on other examples of co-evolution between human culture and canine genomic variants. It is even possible to assume at least partial parallel evolution between the genomes of the two species. To support this hypothesis, a list of genes that have undergone positive selection in the dog's evolutionary history has been compiled, which largely overlaps with the corresponding list of positively selected loci in humans. The most apparent parallel evolution concerns genes involved in digestion, metabolism, neurological processes and cancer. One example of a locus under positive selection concerns a 5 Mb genomic region on chromosome 6. Deletion of the homologous region (carrying the

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[2] Homologous genes are present in different species that have evolved from the same ancestral gene.

GTF2I and *GTF2IRD1* genes) in humans is linked to Williams–Beuren syndrome (WBS), a multi-systemic, disabling congenital disorder, one component of which is hyper-social behavior. However, hyper-sociability is also a central feature of domestication that distinguishes dogs from wolves. Structural variation in the same region in dogs also contributes to extreme sociability. This discovery suggests that there are commonalities between the genomic architecture of the WBS and canine taming, and that directional selection may have targeted a unique set of genes enabling rapid behavioral divergence of dogs and wolves, facilitating coexistence with humans.

A bond involving gaze also exists between humans and dogs. Mutual gaze is thought to increase the release of oxytocin, which in turn increases their propensity to stare at their master, which also has an effect on humans. In contrast, wolves, which rarely make eye contact with humans, seem resistant to this effect. Humans and dogs are locked in an oxytocin feedback loop that is partly mediated by this sustained eye contact.

We can give one last example of parallel evolution. In the same way that the variant of the *EPAS1* gene, inherited from the Denisovan man giving adaptation to life at altitude, was selected in *Homo sapiens* and is found in Tibetan human populations, the *EPAS1* homologue, derived from altitude-adapted gray wolves and has also been selected in the Great Danes that have populated the Tibetan plateau for several hundreds of years.

Understanding how humans and dogs interact is therefore important, as specific human practices and mutual cooperation between the species can explain changes in the physical, behavioral or functional appearance of dogs.

1.4. Selection of modern breeds, evolution in an anthropic context

1.4.1. *Origins and timing of selections*

Today, dogs have many different roles, ranging from protection, hunting and herding to companionship, revealing very different selective pressures over time. Today, in Europe, dogs have no economic value for food production and are mainly companions for humans. With 85.2 million individuals in Europe (25% of families have at least one dog in France or the UK; 42% in Poland or Romania), dogs play a major social role. The process of domestication was one of the first causes of morphological changes at the origin of the current phenotypic diversity of dogs (in addition to those from the wild pool). This has been demonstrated in fox domestication experiments (Trut et al. 2009) as a model (loss of wild color, drooping ears, shortening of limbs, curved tail, wider muzzle, reduction in size, etc.)

and by the archaeological approach (widening of the skull, shortening of the mandible or overlapping of teeth). Since their domestication, the dog's genome has evolved as a result of (i) unconscious or directed human selection for preferential phenotypes, or phenotypes that facilitate domestication; and (ii) the adaptation of animals to variations in their new environment and to their new lifecycles, imposed by chronological and cultural contexts. In the present state of knowledge, the timing and speed of the first human-mediated selections, as well as the traits selected by humans, remain unknown. However, it is possible that voluntary selection of phenotypic traits by humans began early in the dog's evolutionary history. This selection could have been initiated by controlling the reproduction of individuals carrying preferentially selected traits that were well adapted to local conditions and could have been highly dependent on socio-cultural contexts. For example, at the Ust-Polui site (Arctic, Bronze Age), the fact that some dogs were ritually buried, while others were slaughtered, suggests a complex set of beliefs about the dog's place in society. Canines were not considered a homogeneous group at this site. These findings suggest that humans built lasting relationships with individuals they deemed valuable to the community, molding them to their needs and cultural specificities. These diverse practices – whether intentional or not – may therefore have influenced the evolution of the dogs, shaping the physical characteristics and personality traits of the animals that lived alongside these communities.

The emergence of the concept of breed standard, some 300 years ago, eventually led to the exacerbated artificial selection of certain phenotypic traits and the fragmentation of the original domestic populations into over 350 breeds. At the root of this phenotypic variability, analyses of modern dog genomes have revealed a handful of mutations (not found in existing wolves) with significant effects on morphological variation (Plassais et al. 2019), such as coat characteristics, length, size, weight and height variation, ear morphology, coat color and behavior (a nonexhaustive list). As this artificial selection is very recent, there is no way of deducing the phenotypes of ancient dogs and the variability of ancestral genotypes from modern data, as most of the initial genetic and phenotypic variation may have been lost in this recent process.

1.4.2. *Phenotypic and genetic variability*

Understanding and identifying the genetic basis of phenotypic variability in dogs has long been the main focus of genetic, and later genomic, studies. Much of the dog's phenotypic diversity derives from variants that are not present in the wolf, which therefore emerged after domestication. Classical genetic analyses have succeeded in identifying certain genes that cause genetic diseases and discrete phenotypic traits, such as coat color variation. However, the identification of the genetic architecture of complex traits, such as body size and conformation, has only been possible with the development of ***GWAS (genome-wide association study) analyses***. These studies have shown that in dogs, most of the variation in a phenotypic trait is due to variation in just a few genes. This probably reflects the method of artificial selection used to fix specific traits, involving few breeding animals, often from the same litter. In contrast to the mutations responsible for each breed's characteristics, carried by just a few genes, the genetic basis of the divergence between dog and wolf appears to be more complex, involving a polygenic architecture. Few loci in coding areas of the genome appear to be associated with this wolf/dog divergence, suggesting a crucial role for mutations in regulatory areas in the evolution of dog-specific traits. Today, genomic analyses suggest the existence of six to nine main groups of dog breeds. The richness of phenotypic diversity is explained by the incredibly small number of mutations shared between breeds, compared to other domestic species. However, while the dog is the species with the greatest phenotypic variability, with over 350 breeds, genetic diversity within each breed is very low.

1.4.3. *Harmful effects and genetic diseases*

While advantageous genomic regions may have been selected, domestication also has detrimental effects on a genomic scale, as stated at the start of section 3.1. Domestication is associated with multiple bottlenecks, and it is therefore expected that the effectiveness of selection in eliminating harmful mutations will be reduced. This effect is amplified by artificial selection, in the course of which breeds are genetically homogenized, notably through the use of a low number of breeding stock, thereby increasing inbreeding and reducing effective population size.[3] Today, this phenomenon is the main cause of genetic disease in dogs. In dogs, there is a link between the genes involved in Mendelian genetic diseases and the genes located in these selective sweep areas that are responsible for the formation of breeds. This link is significantly higher in dogs than in human populations. This is the case, for example, with selection for merle color, one of the most popular coat patterns in dogs. Mating between merle individuals can result in double merle (MM) offspring with eye defects (including blindness and microphthalmia) and hearing defects. Dog breeders, in selecting phenotypic traits, often unknowingly target mutations present in adjacent regions, resulting in genetic diseases (such as chondrodysplasia,

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[3] The size of a theoretical population which would evolve, from the point of view of inbreeding and genetic drift, with the same speed as the population studied. This theoretical population is closed, of constant size, made up of individuals who reproduce randomly, without selection or mutation.

dwarfism, hereditary cancers, obstructive brachycephaly and dysplasia). Certain phenotypes found in dogs are observed in wild canid populations, such as chondrodysplastic limbs (shortened limbs similar to those of dachshunds, corgis or basset hounds), but they are however counterselected, given their extreme impact, in this case, on movement. Modifications to breeding programs and guidelines must therefore include avoiding inbreeding and ensuring greater variation within breeds, so as to prevent the accumulation and expression of harmful mutations and improve dog health.

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