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NOTAS TÉCNICAS

ANCIENT DNA TO DECIPHER THE DOMESTICATION OF DOG

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The agriculture and farming started about 15,000 years ago in the Middle East. The study of the domestication of plants and animals is not only a fascinating topic, contributing to decipher the history of humankind, but also has basic and applied scientific potential. Thus, understanding the species evolution in general and the domestication in particular will foster the advancement of basic scientific knowledge and assist new breeding developments.

The dog is an emblematic example among the domesticated species, being used not only to protect properties and warn (barking) of approaching animals (including humans), during the day or night, but also to hunt and even as pet. That is why the dog is also known as the “man’s best friend”, which reveals the peculiarity of such domestication, which is in fact matchless. A clear evidence of such significance is the widespread number of modern dog breeds (Parker et al, 2004), which began about 5,000 years ago, leading to as many as 500 breeds. Indeed, such amazing phenotypic diversity was used by Darwin as an evidence supporting his theory of the mutation, adaptation and evolution, explaining the origin of species by natural selection and survival of the fittest.

Genetic analyses point to the wolf (*Canis lupus*) as the wild origin of the dog (*Canis lupus familiaris*). Some knowledge of such process can be obtained from modern DNA analyses, in which the wolf and dog genome projects are undoubtedly shedding new light (Wayne and Ostrander, 2007; DGR, 2009; WGS, 2009). But the ultimate history of the dog domestication comes also from the study of ancient remains from both wild wolves and domesticated wolves (aka, dogs). Modern molecular biology technologies in general, and the so-called “next-generation sequencing” in particular (Dorado et al, 2009) allow to study not only specific genome targets or loci, but even full genomes, much as the Neanderthal (*Homo neanderthalensis*) genome project is demonstrating (Green et al, 2006; Noonan et al, 2006; Dalton, 2009). Thus, both classical (eg., osteometry and stable isotopes) and modern (eg., molecular biology) archaeology are taking such subject in general and the study of the wild wolf domestication in particular to new scientific levels (Vila et al, 1999; Savolainen et al, 2004; Sharma et al, 2004; Dorado et al, 2007, 2008; Horsburgh, 2008; Deguilloux et al, 2009; Dorado, 2010; Germonpre et al, 2009).

Several factors should be considered when studying ancient DNA. Thus, the archaeological remains are usually incomplete, not including all chain links of the evolutionary or domestication process. Beside, the amount of each archaeological specimen is usually very limiting, also being a very precious sample that sometimes is not amenable for analyses requiring destructive analytical methodologies. On the other hand, special care should be taken to prevent the contamination of the ancient samples with modern DNA, which may jeopardize the subsequent downstream analyses. Additionally, the ancient DNA (aDNA) of archaeological remains may be absent, chemically altered and too physically degraded, which may block or at least challenge its downstream processing. Likewise, such aDNA may contain Polymerase Chain Reaction (PCR) inhibitors, preventing the amplification and thus further analyses like agarose gel electrophoresis (AGE) or DNA sequencing (Lario et al, 1997; Dorado et al, 2008).

The diploid cells have two copies of each nuclear DNA (nuDNA) chromosome per cell. On the other hand, such DNA is quite labile, being linear and thus prone to exonuclease degradation. Eukaryotic cells have also cytoplasmic DNA. Depending on cell activity, cell type and species, there are 50 to 100 mitochondria per cell, and each mitochondrion has about 50 to 100 copies of its genome, which yields a potential of 2,500 to 10,000 copies of mitochondrial DNA (mtDNA) per animal cell. Besides, such DNA is circular, and thus resistant to exonucleases, being also protected by the double mitochondrion envelope. Supercoiling of relatively small double-stranded DNA (dsDNA) as the mtDNA does not require proteins like the histones associated with linear DNA, which represents yet another protective element to the ancient mtDNA integrity.

Therefore, the best target for the study of the aDNA from both wild wolves and dogs is the mtDNA. Besides, such DNA has yet another advantage for these analyses: it contains DNA regions that are very conservative, moderately conservative, standard, variable and hypervariable. Being also a rather small chromosome (~16,728 bp for wolves and dogs), it can be easily sequenced and compared among species and breeds. The control (D-loop) and hypervariable regions have been usually chosen as the ideal target for evolutionary and forensic studies (VanAsch et al, 2009), together with other more stable regions like the cytochrome b gene (cytb), as well as nuclear microsatellites or Short Tandem Repeats (STR), and nuclear Single Nucleotide Polymorphisms (SNP).

The question of the origins of the dog has been much debated, ranging from several dog-domestication events in Europe and Asia, to a single one in

East Asia (Verginelli et al, 2005; Dobney and Larson, 2006; Malmstrom et al, 2008). The molecular evidence clearly shows that the dogs evolved by domestication from the wolf (Vila et al, 1997; Leonard et al, 2002). In fact, such domestication took place at the end of the last glaciation, and is now considered a key step leading to the human Neolithic revolution (Verginelli et al, 2005, 2006). On the other hand, some archaeological evidence suggests that the domestication of the wild wolf started in Central Europe 14,000 years ago during the Upper Late Paleolithic (Clutton-Brock, 1995, 1999; Muller, 2002). The first dog fossil remains have been found in Russia, Europe (Germany) and the Middle East at the end of the last glacial period (12,000 to 17,000 years ago). Other authors argue that the wolf domestication was carried out 33,000 years ago (Hirst, 2009). Since the dogs were not shown on the European Upper Paleolithic art, it is assumed that they were not present at such a time and places (Verginelli et al, 2006). The studies of ancient wolf and ancient dog mtDNA suggest a contribution of European wolves to the three major dog clades, which is in agreement with archaeozoological data (Verginelli et al, 2005, 2006).

Some studies suggest that the domestic dog may have originated from several hundred wolves in southern China less than 16,300 years ago. The site and timing is related to the origin of the rice agriculture. Thus, the wild wolves may have been domesticated by sedentary hunter-gatherers or early farmers. Additionally, wolf taming was probably an important culture trait, as suggested by the different founders found (Pang et al, 2009). Other molecular evidence suggests that the definitive domestication took place in the Near East (Driscoll et al, 2009). Yet another results are in disagreement, inconclusive or spell some doubts about the interpretation of the results, so the wild wolf domestication origin remains a mystery. Thus, it has been found that modern East Asian dogs exhibit the highest mtDNA diversity, suggesting an East Asian origin of domestication (Savolainen et al, 2002), yet such result may be a consequence of high levels of mitochondrial diversity in the studied dogs and not necessarily an indication of East Asian domestication (Pires et al, 2006). In fact, in a recent study of mtDNA, nuclear microsatellites and nuclear SNP markers from modern dogs, it has been recently concluded that mtDNA alone may not be enough to determining the timing and location of domestication. Thus, in the absence of multiple highly diverged and highly localized mitochondrial haplogroups, autosomal markers across the nuclear genome may be required to unravel the story of the first domesticated species, potentially calling into question the hypothesis of an East Asian origin for the wild wolf domestication (Boyko et al, 2009).

All this controversy is also related to a high variability in the nuclear genes and the possible retro-crosses with wild wolves, introducing additional genetic diversity within already domesticated dogs, after the original domestication event. Thus, such introgressions of wild genomes cannot be traced studying the mtDNA if such wild wolfs were males (which is the most likely scenario), due to the maternal inheritance of the mtDNA (Ellegren, 2005). Not surprisingly, some current dog breeds are barely distinguishable to the naked eye of the wolf, and even in character and social behavior some dogs and wolves are quite similar. It is obvious that the wolf domestication is a work in progress.

In summary, the domestication of the wild wolf to become the modern dog is yet another example of the importance of the classical and modern archaeological studies to decipher the molecular keys underlying the evolutionary processes, with important scientific implications for both the basic knowledge advancement and the application of such discoveries for breeding programmes.

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