VIEW POINTS
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Strengthening Ancient mtDNA Equid sequences from Pompeii

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Dear Editor,

I am aware of the manuscript published by your Journal on December issue (111(5):1080-1081) from Susan M. R. Gurney, Institute of Continuing Education, University of Cambridge, entitled: "Revisiting ancient mtDNA equid sequences from Pompeii".

The author reports our mtDNA studies [Di Bernardo et al., 2004a,b] on six equine mtDNA sequences labeled CAV1-5 and CAVH, respectively, recovered from the ancient Roman towns of Pompeii and Herculaneum, buried by the Vesuvius eruption in AD79. We stated that "our findings provide evidence that the remains analyzed are those of horses and mules and do not include either donkeys or hinnies" [Di Bernardo et al., 2004a] and that "the peculiar sequence polymorphisms shown by CAV5 could suggest to belong to a haplotype, which has either not yet been documented in GenBank or has since disappeared."

Dr. Gurney suggests that in CAV5 analysis "we have inadvertently hybridized a horse mtDNA sequence with an ass mtDNA sequence" just because the first 177 nucleotides match closely with ass sequences in Genbank while the second section of 193 nucleotide matches closely with horse sequences in Genbank. She also says "these two sections correspond exactly to the two different PCR: primer pairs that the authors used."

First of all, I would like to point out that our experimental procedures in carrying out PCR on ancient DNA strictly obey to well established guidelines to avoid contamination [Cooper and Poinar, 2000; Hofreiter et al., 2001].

In particular, as reported in the Material and Method sections of our papers, at least two DNA extractions, carried out by different operators, and using different parts of the intact long bone, were taken for each bone sample. Moreover, each DNA sample was subjected to independent PCR amplification. DNA extraction and pre-PCR and post-PCR steps were carried out in physically separated laboratories, all surfaces and instruments were bleached, and plastic ware and solutions underwent UV irradiation. The operators wore fresh body protection for each pre-PCR step, and wore gloves and face masks throughout the entire pre-PCR and PCR work. Each PCR included two negative controls, i.e., a PCR control and an extraction control. The bone was first cleaned with a brush to remove the outer layers and surface contamination. Fragments excised with sterile scalpel blades were treated with highly concentrated bleach, and were then exposed to UV light for 15 min at a distance of 20 cm and powdered in a grinding mortar. DNA was extracted from ancient samples using a previously reported procedure [Hoss and Pääbo, 1993; Lambert et al., 2002] with slight modifications.

This experimental procedure excludes the possibility that starting material for DNA extraction was a mixture of bone powder from different animals.

Moreover, our data on the 16SrRNA mtDNA gene [Di Bernardo et al., 2004a,b], showing that CAV5 exhibited a specific horse polymorphism in a Mae III restriction site, further support the observation that the CAV5 bone that we used to prepare powder extracts was not contaminated with a mixture of different animals.

We would also reject another of Gurneys' statements. She supposed: "this hybrid sequencing artifact can be explained by the fact that the two primer pairs overlap only slightly and can recognize both horse and ass mtDNA." In fact, she says that the Herculaneum horse sequence is identical to the horse section CAV5 except for a single nucleotide difference. She also says that: "This single nucleotide difference is, however, close to the internal primer." We would like to draw your attention to the fact that the single nucleotide difference between CAV5 and CAVH was confirmed by sequencing the amplicon on both strands in different DNA extracts. While on a strand, the position of the polymorphic nucleotide is near the primer EQCR230, as indicated by Gurney, on the complementary strand it is located 161 nucleotides further away from the primer EQCR19, thus excluding a sequencing artifact despite the presence of a slight overlap between amplicons. Finally we would like to underline that the primers were intentionally designed to match equine mtDNA and that the subsequent nucleotide sequencing allowed to distinguish among equine species on the basis of specific single base polymorphisms.

Finally, we would like to thank Dr. Gurney for her consideration of our study.

Yours sincerely,

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